STUDIES ON SOME SALIGENIN CYCLIC PHOSPHORAMIDOTHIONATES HAVING FUNGICIDAL ACTIVITIES

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A C K N O W L E D G E M E N T

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Darjeeling. 1982

S Y N O P S I S

The work embodied in this dissertation is related to the investigation of some 2-alkylamido -6- nitro - 4H - 1,3,2 - benzodioxaphosphorin - 2 - sulphides with reference to their chemical, insecticidal, fungicidal, biochemical, phytotoxic and other toxicological properties besides structural elucidations by chemical analyses and spectroscopic methods.

PART - I:

In Part - I of this thesis, a general introduction of Organophosphorus pesticides including fungicidal activities, anticholinesterase and hydrolytic properties have been presented.

PART - II:

Part - II of this dissertation has been devoted to a short review describing chemical, fungicidal, insecticidal, biochemical, toxicological, hydrolytic, anticholinesterase and other properties of saligenin cyclic phosphorus compounds with special emphasis on salithion (2-methoxy-4H-1,3,2 - benzodioxaphosphorin - 2 - sulphide). It has been revealed that the biological activities of these compounds are greatly influenced by the exocyclic substituents on the phosphorus atom, and also by the substituents in benzene ring or in hetero-cyclic ring.

PART - III:

Part - III deals with the work related to the synthesis and structure determination of some 2-alkylamido-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-sulphides. These compounds have been synthesized by the reaction of the corresponding phosphoramidothioic dichlorides with 5-nitro-saligenin.

The structure of the compounds have been determined by chemical analysis and IR, mass and PMR Spectra.

The common IR bands are:

1010 - 1030 cm^{-1}(s), P-O-C (alkyl); 1235-1250 cm^{-1} (vs), P-O-C (aryl); 880-920 cm^{-1}(s), P-O-C (aryl); 1515-1520 cm^{-1}(s), asym. str. of nitro group; 1340-1345 cm^{-1} (s), sym. str. of nitro group; 800-820 cm^{-1}, P = S (I); 640-660 cm^{-1}, P=S (II).

The compounds show parent molecular ion (M^+) peaks in the mass spectra. Fragmetation by loss of 'SH' radical is important; all compounds show an ion due to $(M - SH)^+$, and it is the base peak for all of the four alkylamidophosphorothionates. Finally the structure of the compounds has been settled by taking PMR spectra; for all compounds the endo - cyclic $-CH_2-$ group gives eight lines in PMR spectra.

The dimethylamido and the morpholino compounds have some insecticidal activity; but their activities are less than that of salithion. The other compounds are non-insecticidal. All compounds are less toxic to rats than salithion.

It has been observed that for any compounds (BD.10 to BD.14), the housefly-head acetylcholinesterase (HFAChE) is more inhibited than the blood - cholinesterase (blood-ChE).

From the chemical hydrolysis studies it has been observed that the compounds containing the di-substituted amido groups are extremely resistant to hydrolysis compared to other compounds having the mono-substituted amido groups. It has also been observed that the rate of alkaline hydrolysis is increased as the p^H value increases from 7.7 to 11.8.

Wheat seed germination studies indicate that none of the compounds are phytotoxic properties upto 600 ppm concentration. Treatments of rice seeds with pyrrolidino and nonylamido compounds have no effect on germination, but root and shoot growth have been reduced drastically; treatments of rice plants with these two compounds have reduced the shoot growth.

From the fungicidal activity studies (by growth inhibition) against P. oryzae, V. albo-atrum, A. solani and H. oryzae indicate that some of the compounds show good inhibitory effect on the growth

of different fungi, however, compared to Hinosan they have less inhibitory effect. Among the nine compounds, the dimethylamido compound is most active against P. oryzae, the isopropylamido compound is against H. oryzae, and the cyclohexylamido compound is against V. albo-atrum & A. solani.

From the spore germination inhibition studies against A.niger, P. sensani, V. albo-atrum and H. oryzae, it has been observed that all compounds are effective. The nonylamido compound is most active for A. niger, and the dimethylamido compound is against P. sensani & V. albo-atrum; their activities are greater than that of Hinosan. The isopropylamido compound is most effective against H. oryzae; but, its activity is less than that of Hinosan.

Protectant activity studies (in vivo) against H. oryzae on detached rice leaves and rice plants by using only two compounds (BD-15 and BD-17) indicate that the activity of nonylamido compound (BD-17) is greater than that of the pyrrolidino compound (BD-15). Protectant activity studies for other compounds have not yet been performed.

The antifungal activity data justify further examination of these phosphoramidothionates as potential fungicides with special reference to the selectivity of their action. Whether the use of these compounds will protect the plants from diseases in the field

remains to be studied.

In order to find out the chemical structure - biological activity relationship in these nitro - saligenin cyclic phosphoramidothionates, we have to synthesize several new compounds in which the nitro group is to be incorporated in different position of the aromatic ring, and to investigate their biological activities phytotoxic properties, fungicidal activities, anti - ChE enzyme activities, and other toxicological properties including delayed neurotoxicity in hens.

Part - I

C O N T E N T S

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ORGANOPHOSPHORUS PESTICIDES
GENERAL INTRODUCTION

Part-II

REVIEW ON SALITHION
AND RELATED COMPOUNDS

Part - III

A. MATERIALS AND METHODS

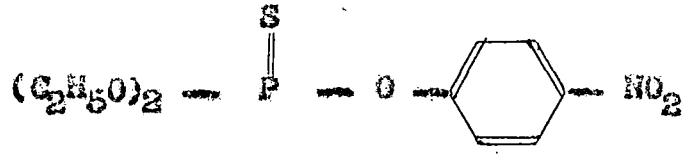
P A R T - I

ORGANOPHOSPHORUS PESTICIDES : GENERAL INTRODUCTION

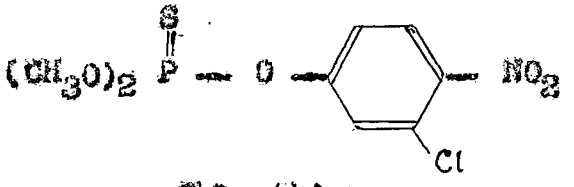
1. INTRODUCTION:

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 bio-degradability, 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, anthelmintic, insect sterilizing, fungicidal, harbicidal, rodenticidal and other purposes. The development of new phosphorus compounds was for a long time dominated almost exclusively by one single guiding principle namely the "Acyl Rule" of Schrader[1,2,3]. The great advancement in agricultural practice, scientific knowledge of the structure activity relationship and mode of action of organophosphorus pesticides were achieved by the discovery of parathion by Schrader in 1944. Parathion is extremely toxic to mammals as well as to insects. Many less toxic pesticides have been synthesized by slight structural modification of parathion, for example, chlorthion (in 1952), fenthion (in 1958) and

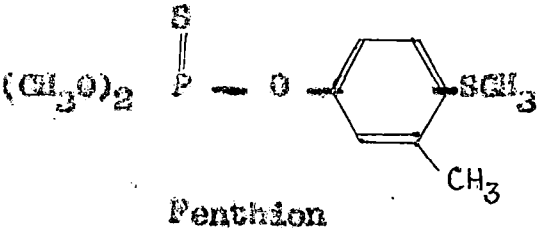
fenitrothion (in 1959) were discovered[3].



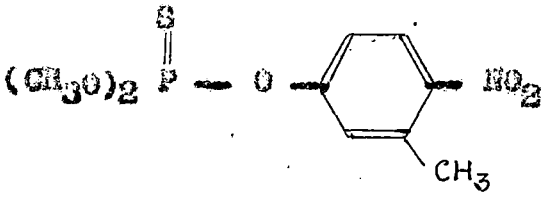
Parathion



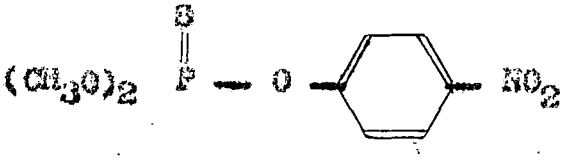
Chlorthion



Penthion

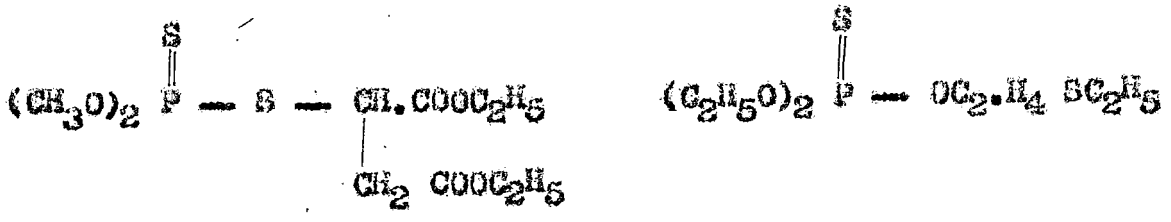


Fenitrothion



Parathion - methyl

Malathion was discovered in 1950 and demeton in 1951.



Malathion

demeton - S

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

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[4-6]. It is only recently they have been gaining importance in the control of pathogenic fungi[2,3]. In comparison to the heavy metal fungicides, the organophosphorus compounds are particularly

favourable as regards to residue problem.

The simplest organophosphate is trimethyl phosphorothioate.

$$(CH_3O)_3 . PS$$

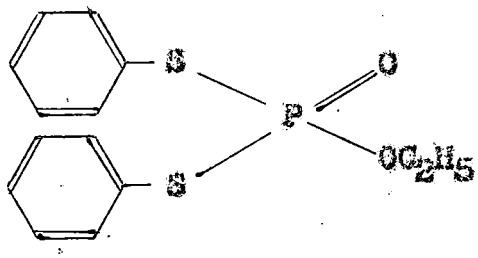
It is an effective, selective soil fungicide to control Pythium sp[7].

Trimethyl phosphorotetrathioate appear to be useful for the control of Pythium sp.[7b,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[7c]. On the other hand, an analogous compound, O,O-diethyl S-methyl phosphorodithioate, is a good fungicide against Rhizoctonia solani[7b]. The fungicidal activity of different other compounds are given below:

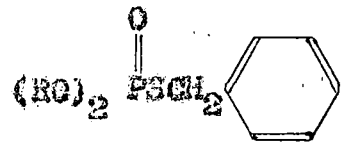
Edifenphos (Hinosan):



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 ester edifenphos, but the methyl and butyl homologs are much less active than the later. The introduction of a chlorin atom into the benzene ring causes a remarkable decrease in fungicidal activity.

Kitazin:



R = $(CH_3)_2$ CH Kitazin P.

R = C_2H_5 Kitazin.

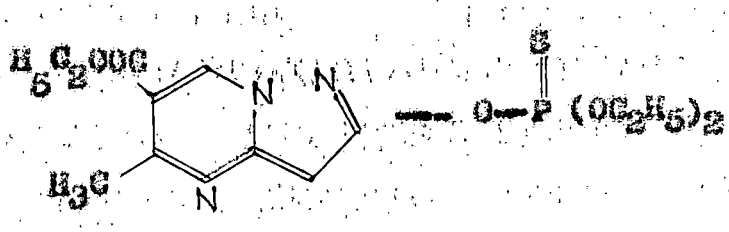
S - Benzyl diethyl phosphorothiolate was first introduced in 1965 as a fungicide under the trade name Kitazin, but was replaced in 1967 by the isopropyl homolog Kitazin P for commercialization.

It is a systemic fungicide used to control Pyricularia oryzae in rice. It is applied at 400 - 600 g a.i. (as e.c.) in 1000 l/ha as soon as the blast lesions appear. One or 2 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 prophylactically.

In the homologous series of dialkyl S-benzyl phosphorothiolates, the maximum fungicidal activity is obtained when the number of carbon atoms in the alkyl group is three or four. The dimethyl homolog has poor activity. In the analogous series, the phosphorothiolate esters are much more effective than corresponding phosphorothionate, phosphorothiolothionate, and phosphate esters. Introduction of substituents such as chlorine atom or

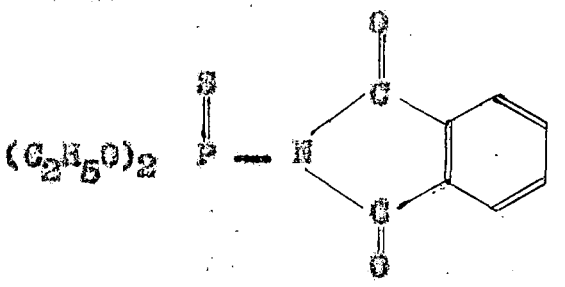
nitro group on the benzene ring has little effect on the increase of the fungicidal activity.

Pyrazophos (Afugen):



Pyrazophos is a systemic fungicide controlling powdery mildews on a wide range of crops at 10 - 30 g a.i./100 l, 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):

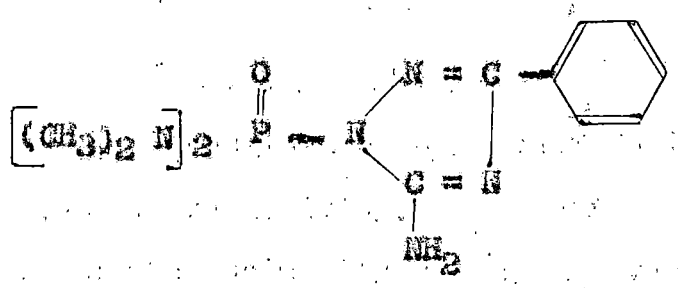


Ditalimfos is a non-systemic foliar fungicide with protectant and curative activity. It is used to control powdery mildew on ornamentals (primarily roses) and vegetables (cucurbits) under glass, as well as under field conditions, at 30 - 50 g. a.i./100 l; on apples (25 - 50 g/100 l) and cereals (500 - 550 g/ha). It is also used against Venturia inaequalis on apples at 37.5 - 100g/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.

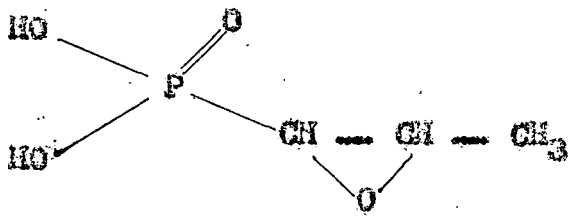
Triamiphos (Wepsyn, Wepsin):



Triamphos 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.

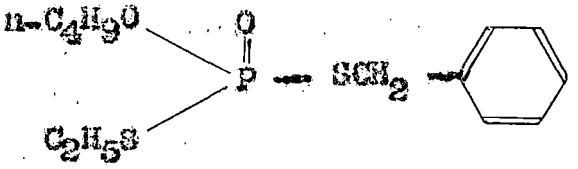
The 5- anilino-3-alkyl analogs of Triamiphos are also active as fungicides.

Phosphonomycin:



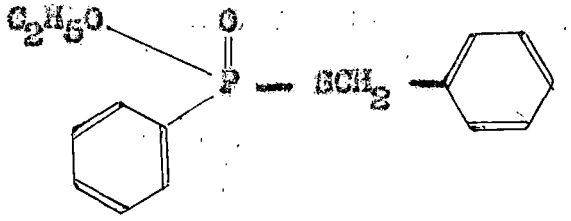
Phosphonomycin is a naturally occuring phosphonate antibiotic recently discovered by Merck & Co. Inc. It was isolated from fermentation broths on which Streptomyces fradiae was grown. Its structure was demonstrated by synthesis[8]. This new antibiotic has a broad spectrum of activity and inhibits irreversibly pyruvate-uridine diphospho-N-acetylglucosamine transferase in extracts of gram-positive and gram-negative micro-organisms. It compares favourably with tetracycline and chloramphenicol.

Conen:



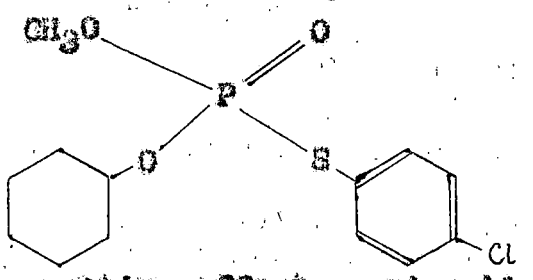
It is a fungicide to control rice blast disease.

Inezin:



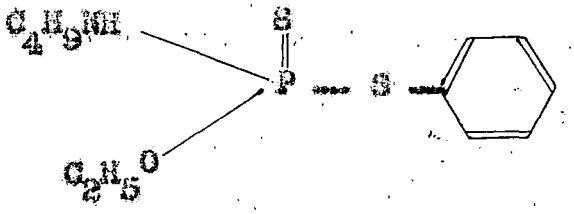
It is a fungicide to control rice blast and rice sheath blight.

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.

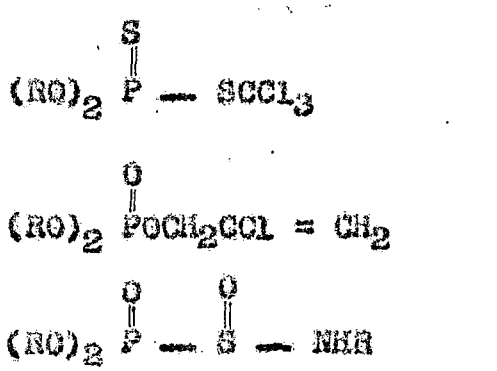
Phosbutyl:

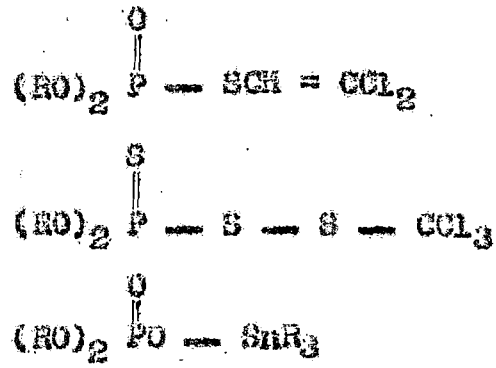


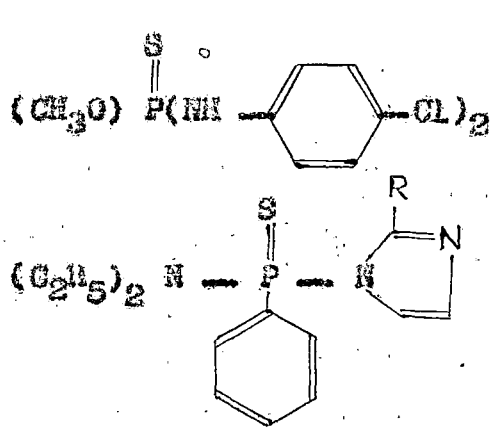
It is highly active against mycelial cells, but not active against

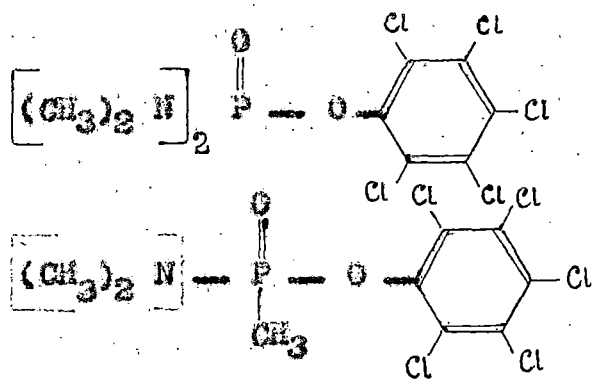
spore germination. Being absorbed rapidly by the plant, it thus shows a good curative activity for many plants infected with pathogenic fungi.

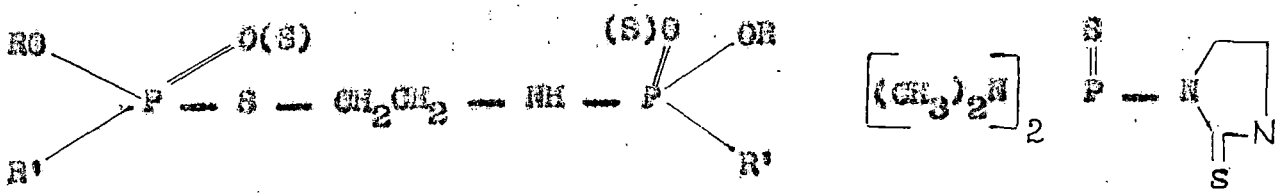
Certain organophosphorus compounds are known to have fungicidal activity. Structures of some selected compounds are given below:











There are certain reviews on organophosphorus fungicides (7b,9,10). There is an interesting correlation among the alkylating activity,

the inhibitory activity against 'SH enzymes', and the antifungal activity of some cyclic organophosphorus esters[11]; many fungicides are known as the inhibitors of 'SH enzymes'[12].

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[3]. The enzyme mainly affected is accepted to be cholinesterase, an enzyme that plays a vital role in hydrolysing acetylcholine. The reaction between acetyl choline (ACh) and cholinesterase (ChEH) takes place in three stages:

$$(CH_3)_3 \overset{+}{N}C_2H_4O - \overset{O}{\overset{\|}{C}} - CH_3 + ChEH \underset{K_{-1}}{\overset{K_1}{\rightleftharpoons}} (CH_3)_3 \overset{+}{N}C_2H_4O - \overset{O}{\overset{\|}{C}} - CH_3/ChEH$$

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.

$$(CH_3)_3 NC_2H_4O - \overset{O}{\overset{\|}{C}} - CH_3/ChEH \xrightarrow{K_2} (CH_3)_3 NC_2H_4OH$$

Enzyme Complex — Choline + CH_3CoECh 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 (Fig. 1). The reaction between an organophosphorus compound and AChE is represented in Fig. 2. 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[13] investigated the inhibition of cholinesterase 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.;

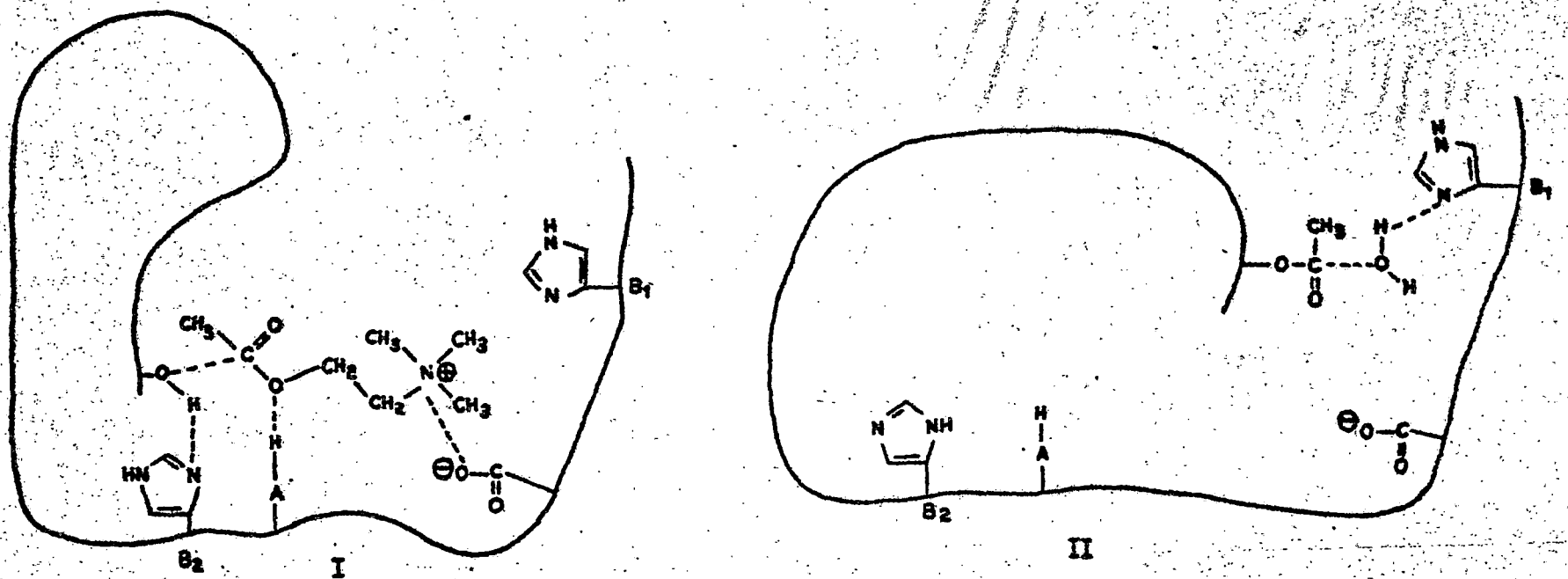


Fig. 1. Schematic Mechanism of action of AChE, after Krupka.
(I) Enzyme - substrate complex in AChE.
(II) Deacetylation of acetyl - AChE.

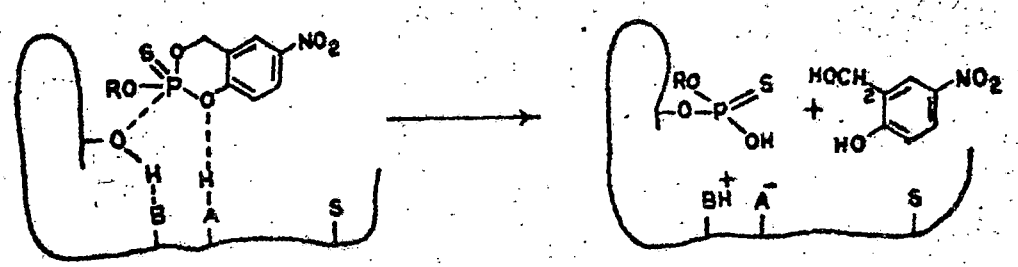


Fig. 2. Schematic mechanism of reaction of organophosphate with AChE.

$$K = \frac{1}{tI} \cdot I_n \frac{100}{b}$$

Where, K = bimolecular rate constant

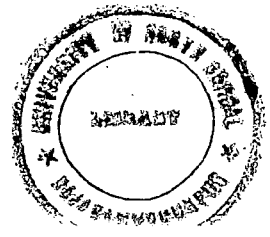
t = time in minutes,

I = molar inhibitor concentration,

and, b = percentage residual activity

Correlation between the reactivity of a organophosphorus compound and its cholinesterase inhibition, however, has not been ideal, and Main[14] introduced a kinetic treatment for the reaction that takes into account the reversibility of the complex. This reversibility is dependent on the affinity of the inhibiting compound for the active site of cholinesterase as well as on the rate of phosphorylation (Fig. 2). By utilizing different kinetic methods the values for K_1 (affinity constant), K_p (Phosphorylation constant), and K_3 (bimolecular inhibition constant) may be determined[12,15].

If the acelylcholinesterase is destroyed, is irreversible 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 products may be obtained from kinetic studies. Hydrolysis rates of these compounds and their metabolites are of interest since chemical hydrolysis determines whether or not toxic residues will persist. The first-order half-lives of some common organophosphorus pesticides [redacted] including some metabolites are listed in Table - 1 (16).

Table - 1

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

Compound	Half-life hours	Compound	Half-life hours
Thimet oxon	0.50	Demeton-S	18.0
Dichlorvos	1.35	Morphothion	18.4
Thimet	1.75	Baytex	22.4
Trichlorphon	3.2	Vamidothion	25.4
Mecarbam	5.9	Menazon	27.6
Maleoxon	7.0	Paraoxon	28.0
Demeton-S-methyl	7.6	Thionazin	29.2
Malathion	7.8	Disulfoton	32.0
Thionazin-oxon	8.2	Diazinon	37.0

.....Contd

Table-1 (Contd.)

Compound	Half-life hours	Compound	Half-life hours
Parathion methyl	8.4	Ethion	37.5
Fenchlorphos	10.4	Parathion	43.0
Azinphos methyl	10.4	Phenkapton	92.0
Sumithion	11.2	Chlorfenvinphos	93.0
Dimethoate	12.0	Carbophenothion	110.0
Thiometon	17.0	Dimefox	212.0
Methyl Oxy-demeton	17.1		

The hydrolysis rate is dependent upon the chemical structure and reaction conditions such as pH, temperature, the kind of solvent used, and the existence of catalytic reagents [3]. In aqueous solution, between the pH range 1 to 5 many organophosphorus pesticides are most stable [117], and in this range (pH 1 to 5), the variation in pH of the solution has practically no effect on the hydrolysis rate. But the hydrolysis rate increases steeply at pH higher than 7, and all organophosphorus pesticides are much more unstable under alkaline conditions. Very good discussions on chemical structure and hydrolyzability of various organophosphorus pesticides are given by Eto [3] and Faust [16].

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P A R T - II

PART - II

REVIEW ON SALITHION AND RELATED COMPOUNDS.

1. INTRODUCTION:

Discovery of saligenin cyclic phosphate as a biologically active metabolite of tri-o-cresyl phosphate (TOCP)[1,2,3] has led to the extensive studies on synthesis, chemical and biological properties of many related compounds[4,5]. Analogous cyclic phosphorus esters have been synthetically prepared for examination of their chemical properties[6,7] and biological activities[8,9]. The biological activities are not always coincident with the chemical reactivities and appear to be influenced by the size of an exocyclic substituent on the phosphorus atom. The TOCP-metabolite causes ataxia in hens but has no insecticidal activity, while its analogous cyclic phosphates carrying a small alkyl group have insecticidal activity[10]. Among the saligenin cyclic phosphorus compounds, salithion (2-methoxy-4H-1,3,2-benzodioxaphosphorin-2-sulphide) has been prepared in a large quantity and practically used as an insecticide in Japan[1,5].

This review describes how salithion has been discovered and developed as practical insecticide and also the chemical, pesticidal, biological and other properties of

saligenin cyclic phosphorus compounds have been discussed.

2. DISCOVERY OF SALITHION AND RELATED COMPOUNDS:

It was the year 1930, when about 10,000 people in USA were stricken with a flaccid paralysis of the lower limbs about 10 days after drinking an adulterated fluid, extract of Ginger [11]. This was due to the phosphate tri-ester of ortho-cresol, so called TOCP, a contaminant present in the ginger extract. Also a similar big outbreak of paralysis took place in 1959 (in Morocco) from cooking oil contaminated with the lubricating oil of turbo-jet aircraft engines [12]. This was also due to o-cresyl phosphate (TOCP) poisoning. The phosphate tri-esters of cresol have been widely used in industries as plasticizers, lubricants, solvents, oil additives and fire-retardants.

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 [13,1] observed that all neurotoxic triaryl phosphates except tri-p-ethyl phenyl phosphate have at least one alkyl group carrying the α-hydrogen atom on the ortho position. This structure-neurotoxicity relationship of triaryl phosphates is clearly understandable by the isolation and characterization of the active metabolities of TOCP [2,3]. The main active metabolite (M) is ortho-tolyl saligenin cyclic phosphate (2-ortho-

4H-1,3,2 benzodioxaphosphorin-2-oxide). It is extraordinarily active in all the biological properties shown by TOCP; this compound (M) is about 100 times more potent to cause ataxia in hens than TOCP: (M) potentiated the toxicity of malathion 100 times by the dose of 20 mg/kg in mice, while TOCP 4 times by the dose 100 mg/kg; (M) is also ten million times more active than TOCP in the in vitro inhibition of plasma cholinesterase[4].

The conversion of TOCP into the cyclic phosphate proceeds via two steps[14] as shown in Fig. 1 : (i) the hydroxylation of the methyl group of TOCP by the mixed-function oxidases (mfo) and (ii) the cyclization by intra-molecular transphosphorylation of the intermediate, di-o-tolyl o-(α -hydroxy) tolyl phosphate, leaving one molecule of cresol. The later reaction takes place slowly in spontaneous manner and is greatly accelerated by the presence of plasma albumin[15].

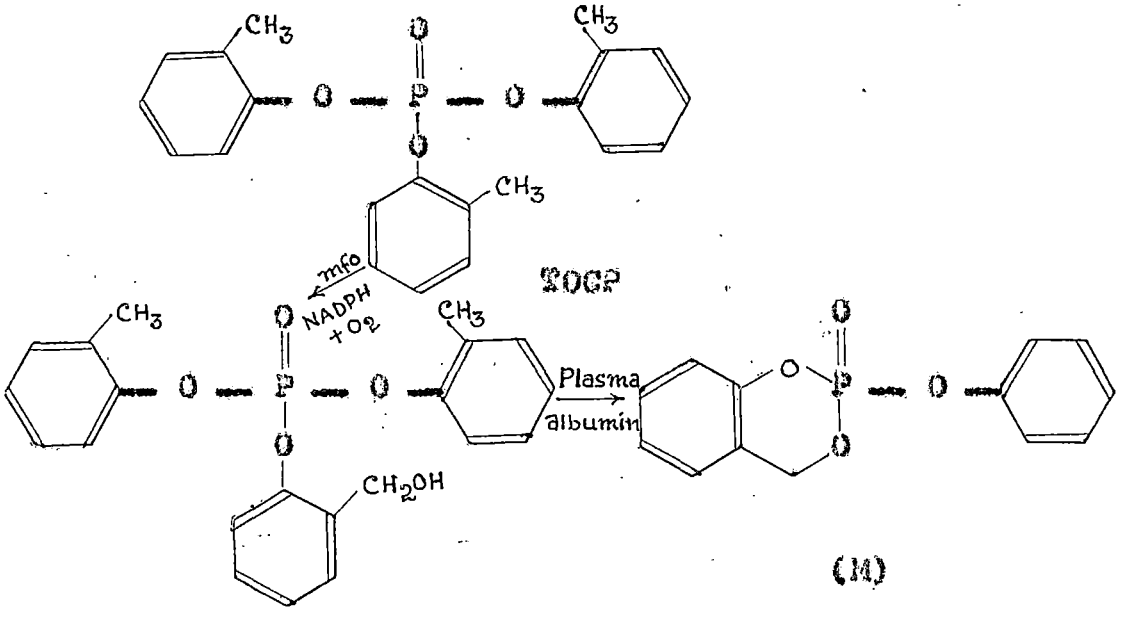


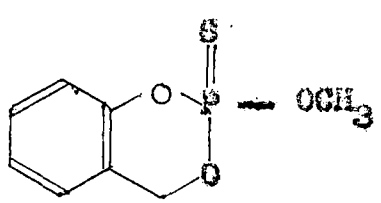
Fig. 1 Metabolic activation of TOCP.

Thus it is rational to presume that the triaryl phosphates having an o-alkyl group with the α-hydrogen atom may be similarly metabolized to give the corresponding active cyclic esters. In the cyclization reaction, no alkyl ester group participates as the leaving group[15]. Actually, no aryl but alkyl saligenin cyclic phosphate is formed *in vivo* from alkyl di-o-tolyl phosphates[16]. Such metabolic activation of TOCP or its analogs have been observed in rats[14], hens[14], cats[17] and insects[16].

All aryl saligenin cyclic phosphates have showed no insecticidal activity but manifested a high delayed neurotoxicity

to cause ataxia in hens; surprisingly the corresponding cyclic esters (both P = O and P = S compounds) having a small alkyl group on phosphorus revealed high insecticidal activity [2,8].

As a result of the aforesaid research "Salithion" (2-methoxy-4H-1,3,2-benzodioxaphosphorin-2-sulphide), an organophosphorus insecticide having a unique cyclic ester structure was discovered by the pesticide research group of Kyushu University [10] in 1963. Salithion was developed into a commercial insecticide in 1968 by Sumitomo Chemical Co. With the co-operation of Toa-Noyaku Co. (now Kumiai Chemical Co.) and Mikasa Chemical Co. of Japan.

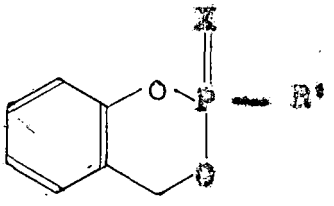


Salithion

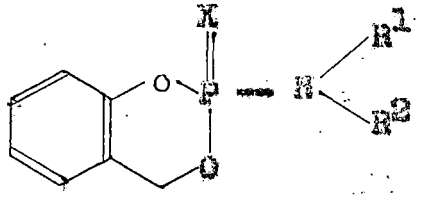
3. SYNTHESIS OF SALIGENIN CYCLIC PHOSPHORUS ESTERS:

The cyclic phosphate and phosphonate esters of saligenin are readily synthesized by condensation of saligenin and substituted phosphoryldichlorides in the presence of a dehydrochlorinating agent such as tertiary amine in a dry solvent like chloroform or toluene at low temperature[6]. In some cases where the reaction is affected difficultly by using the tertiary amine, the reaction has been made to proceed by heating the mixture for 10 to 20 hours in the presence of anhydrous potassium carbonate together with copper powder[18] instead of tertiary amine.

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



(I)



(II)

The process using potassium carbonate is made to proceed by a reaction between liquid and solid phases. Therefore, even if potassium carbonate is employed as finely divided powder form, it often causes a remarkable lowering and fluctuation of the yield[18]. Thus salithion was first prepared with inconsistent and, often, very low yield by heating (90°C) saligenin and methyl phosphorodichloridothionate in toluene for a long period (more than 15 hours) in the presence of anhydrous potassium carbonate together with copper powder as catalyst[19].

This difficulty, has been however, overcome later by applying the well-known Schotten-Baumann acylation procedure using an aqueous solution of sodium hydroxide (Fig. 2)

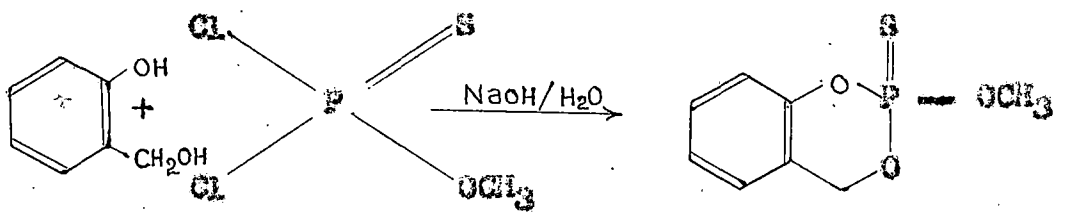


Fig. 2 Synthesis of Salithion.

Some typical examples are given below:

(a) Salithion (2-methoxy-4H-1,3,2-benzodioxaphosphorin-2-sulphide[18]:

6.2 gm o-hydroxy benzyl alcohol was dissolved in 30-40 ml. 20 percent (by weight) sodium hydroxide aqueous solution. Methylthiophosphorodichloridate (8.3 - 11 gm) was then added dropwise to the above mixture at about 10°C with constant vigorous stirring. After the addition, stirring was continued for one hour, as a result, crystals were separated. 70 ml of chloroform or toluene was added to the reaction mixture and the stirring was continued for additionnl one hour. The organic layer was separated and washed with 2% sodium hydroxide, 0.5 (N) HCl and water, and dried over anhydrous sodium sulphide; the solvent was then removed at reduced pressure, and the solids thus separated, were recrystallized from methanol to give 6.5 - 7.6 gm (yield 60 - 70 percent) pure crystals of salithion, M.P. 52°C.

Methylthiophosphorodichloridate can be prepared by the reaction of CH_3OH and $PSCl_3$ in presence of a base[20a,b].

(b) 2-alkoxy-6-nitro-4H-1,3,2-benzodioxaphosphorin 2-sulphide[21,22]:

These compounds were prepared by adding 1 mole 5-nitro saligenin in dried acetone dropwise to a mixture of alkylthiophosphorodichloridate (1 mole) and anhydrous potassium

carbonate (2 moles) in acetone at -5° to + 5°C with constant vigorous stirring. After an additional stirring period of 1 - 3 hours at 0° - 5°C, the solids were filtered out of the reaction mixture and the solvent was removed at room temperature. The residual solid mass was dissolved in ethyl acetate and washed with ice cold water containing 5% sodium chloride. The ethyl acetate phase was lyophilised and the solvent was removed at reduced pressure. For methoxy compound a semi-solid deep-brown paste was obtained; after standing for several days, the crude mass was solidified, and the solid, after washing several times, was recrystallized from methanol. All other compounds were solids [21].

(c) 2-alkylamido-4H-1,3,2-benzodioxaphosphorin-2-sulphide/oxide [23]:

Saligenin cyclic phosphoramidates and phosphoramidothionates were synthesized from saligenin and appropriate phosphoramidic dichloride by the action of proper dehydrochlorinating agent. The reaction of saligenin with reactive dichloride as mono-alkyl phosphoramidic dichloride and some others was proceeded by the action of tertiary amine such as pyridine or triethylamine in cold condition. Liquid dichloride was usually added dropwise to the mixture of saligenin and the base in chloroform. Solid dichloride was however, dissolved in cold chloroform with saligenin, then the amine was added dropwise to the chilled mixture. When the dichloride was less reactive as

almost all phosphoramidothionic dichlorides, heating the mixture of reactants in toluene in the presence of anhydrous potassium carbonate and copper powder was useful. Typically, 2-methylamino-4H-1,3,2-benzodioxaphosphorin-2-oxide was prepared in the following procedure : To a mixture of saligenin, 9.2 gm, triethylamine, 15 gm, and 90 ml. chloroform, was dropwise added 12 gm methylphosphoramidic dichloride with stirring and cooling in an ice bath. After the completion of addition, the reaction mixture was kept overnight at room temperature, and then washed sequentially with ice-water, dil. HCl acid, aq. sodium bicarbonate solution and ice-water. The solvent was removed in vacuo after drying over anhydrous sodium sulphate. Crude crystals were recrystallised from benzene to yield pure crystals, m.p. 87°C (8 gm). Phosphoramidic and phosphoramidothionic dichlorides were prepared according to Michaeli's[24] method.

(d) Saligenin Cyclic Phosphorothiolates[25] :

Phosphorodichloridothiolates reacted with saligenin in the presence of solvent and pyridine or other tertiary amine at room temperature or at about 50°C. The products were purified by distillation in vacuo or recrystallization. Typically, 2-Methylthio-4H, 1,3,2 benzodioxaphosphorin-2-oxide (MTBO) can be prepared in the following procedure:

To a mixture of saligenin (6.2 gm), pyridine (8 gm) and chloroform (100 ml) was added dropwise methyl phosphorodichloridothiolate (8 gm) with stirring at 20°C. After stirring for three hours, the reaction mixture was washed in sequence with water, dil. alkali, dil. HCl acid and water and dried over anhydrous sodium sulphate. The solvent was removed under reduced pressure and the residue was distilled *in vacuo* to give 3.2 gm of oil [b.p. 144 - 145°C (0.1 mm Hg)]. The oil solidified slowly at room temperature (m.p. of pure MTBO 44°C).

MTBO can also be synthesized from salithion by the following procedure (25) :

A mixture of salithion (20 gm, 0.0928 mole), potassium iodide (3 gm, 0.018 mole), methyl iodide (9 ml, 0.145 mole) and dimethyl formamide (20 ml) was kept at temperature 25°C for 24 hours. The mixture was then carefully concentrated to avoid complete dryness *in vacuo* and dissolved in chloroform (50 ml). Chloroform solution was washed with water (4 times) and dried over anhydrous sodium sulphate. After filtration, the filtrate was evaporated at reduced pressure to give MTBO (8.95 gm) in 45% yield. The residue was solidified on cooling. MTBO was recrystallized from ethanol at -15°C.

2-substituted-4H-1,3,2-benzodioxaphosphorin-2-sulphides including methoxy (salithion), alkylamino, alkylthio and

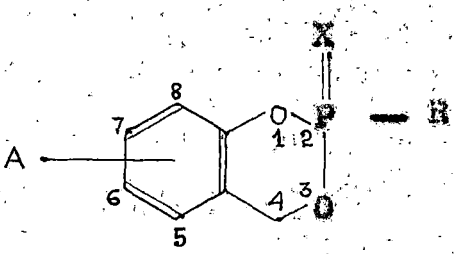
arylthio derivatives were synthesized in good yield by the application of Schotten-Baumann acylation reaction (procedure 'a' given above); this method was very useful for the preparation of cyclic esters which were difficultly prepared by a tertiary amine method[27]. But, 6-nitro derivatives can be best prepared by the procedure 'b' mentioned above[21,22].

4. Other Saligenin Cyclic Phosphorus Esters:

The careful observation of literatures[18,19,25,27,28,29,30] furnishes a variety of saligenin cyclic phosphorus esters in good numbers, which have been prepared and examined for insecticidal activity as well as other biological properties. They involve phosphates, phosphorothiolates, phosphoramidates, phosphonates and their thiono-analogs. A comprehensive but not a complete list of saligenin and ring substituted saligenin cyclic phosphorus esters is given in Table-I and Table-II.

Table - I

Substituted Saligenin Cyclic Phosphorus Esters with Physical Properties.



R	A	X	*Procedure	b.p. °C/mm Hg(m.p. °C)
OCH_3	H	S	(S)	55-56°C
OCH_3	H	O	(P)	110-2°/0.05
O-n-C_3H_7	H	O	(P)	129-32°/0.05
O-n-C_4H_9	H	O	(P)	150-4°/0.05
OC_2H_5	H	S	(P)	Liquid (not distilled)
OC_6H_5	H	S	(P)	(30°)
C_6H_5	H	S	(P)	(37°)
CH_3	H	O	(P)	140°/0.5 (35°)
C_2H_5	H	O	(P)	143-9°/0.3 (25°)
i-C_3H_7	H	O	(P)	(80°)
Sec-C_4H_9	H	O	(P)	110°/0.5
t-C_4H_9	H	O	(P)	(74°)

Contd.....

Table-I (Contd.....)

R	A	X	*Procedure	b.p. °C/mm Hg(m.p. °C)
$CH=CH_2$	H	O	(P)	155°/2.5
CH_2Cl	H	O	(P)	160°/0.8 (51°)
CH_2CH_2Cl	H	O	(P)	139-141°/0.1
CH_3	H	S	(P)	130°/0.6
C_2H_5	H	S	(P)	120°/0.6
i-C_3H_7	H	S	(P)	103°/0.6
CH_2Cl	H	S	(P)	146-155/0.4
OCH_3	6-CH_3	O	(P)	139-140/0.3
OC_2H_5	6-CH_3	O	(P)	152-156/0.3
OCH_3	7-CH_3	O	(P)	109/0.05
OC_2H_5	7-CH_3	O	(P)	112-118/0.05
O-n-C_3H_7	7-CH_3	O	(P)	141-147/0.1
C_6H_5	7-CH_3	O	(P)	(93-95)
$NHCH_3$	7-CH_3	O	(P)	(145-146)
OCH_3	8-CH_3	O	(P)	118-120/0.5
OC_2H_5	8-CH_3	O	(P)	165/0.6
OC_6H_5	8-CH_3	O	(P)	135-140/0.6
OCH_3	6-Cl	O	(P)	145-152/0.2
OC_2H_5	6-Cl	O	(P)	160/0.2
O-n-C_3H_7	6-Cl	O	(P)	167-169/0.15
O-n-C_4H_9	6-Cl	O	(P)	137/0.18

Contd.....

Table-I (Contd.....)

R	A	X	*Procedure	b.p. ^oC/mm Hg(m.p. ^oC)
OC_6H_5	6-Cl	O	(P)	(89°)
$NHCH_3$	6-Cl	O	(P)	(148°)
OCH_3	8-Cl	O	(P)	170-171/0.15
OC_2H_5	8-Cl	O	(P)	151/0.18
O-n-C_3H_7	8-Cl	O	(P)	183/0.18
O-i-C_3H_7	8-Cl	O	(P)	137/0.04
OC_6H_5	8-Cl	O	(P)	203/0.52 (54°)
$NHCH_3$	8-Cl	O	(P)	(128-129°)
OCH_3	6-CH_3	S	(S)	(34-35°)
OC_2H_5	6-CH_3	S	(S)	(71-72°)
O-n-C_3H_7	6-CH_3	S	(S)	158-160/0.2
OCH_3	7-CH_3	S	(S)	110-115/0.65
O-n-C_3H_7	7-CH_3	S	(S)	140-142/0.65
OCH_3	8-CH_3	S	(S)	68-70/0.15
OC_2H_5	8-CH_3	S	(S)	108-109/0.15
O-n-C_3H_7	8-CH_3	S	(S)	120-124/0.15
OCH_3	6-C_6H_5	S	(S)	Oil**
OC_2H_5	6-C_6H_5	S	(S)	Oil**
O-n-C_3H_7	6-C_6H_5	S	(S)	Oil**
OCH_3	6-OCH_3	S	(S)	Paste**
OCH_3	6-Cl	S	(P)	170-178/0.2

Contd.....

R	A	X	*Procedure	b.p. ^{o}C/mm Hg(m.p. ^{o}C)
$NHCH_3$	6-Cl	S	(P)	175-180/0.25
SCH_3	6-Cl	S	(P)	160-170/0.2
OCH_3	8-Cl	S	(S)	($72-73^{o}$)
$NHCH_3$	8-Cl	S	(P)	($46-47^{o}$)
SCH_3	8-Cl	S	(S)	Oil**
OCH_3	6-NO_2	S	(S)	Paste**
OCH_3	6-Cl	S	(S)	Paste**
	8-C_6H_5	S	(S)	
OC_2H_5	" "	S	(S)	Paste**
O-n-C_3H_7	" "	S	(S)	Paste**
OCH_3	6-C_6H_5	S	(S)	Paste**
	8-Cl			
OC_2H_5	" "	S	(S)	Paste**
O-n-C_3H_7	" "	S	(S)	Paste**
OCH_3	6,8-Cl	S	(S)	($57-58^{o}$)
OC_2H_5	"	S	(S)	Oil**
$NHCH_3$	"	S	(S)	Oil**
SCH_3	H	S	(S)	($69-70^{o}$)
SC_2H_5	H	S	(S)	145-147/0.2
S-n-C_3H_7	H	S	(S)	145-150/0.25
S-i-C_3H_7	H	S	(S)	140-143/0.1

Contd.....

Table-I (Contd.....)

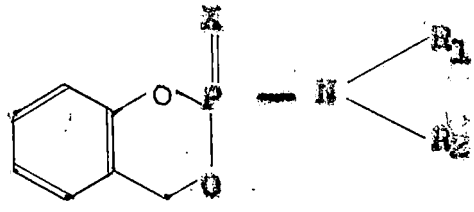
R	A	X	*Procedure	b.p. °c/mm Hg (m.p. °C)
S-C_2H_5	H	S	(S)	140-147/0.3
S-n-C_4H_9	H	S	(S)	160-167/0.25
S-C_6H_5	H	S	(S)	(79-80°)
SCH_3	H	O	(P)	144-145/0.1
SC_2H_5	H	O	(P)	140-145/0.04
S-n-C_3H_7	H	O	(P)	145-147/0.07
S-i-C_3H_7	H	O	(P)	155-158/0.1
S-n-C_4H_9	H	O	(P)	157-160/0.02
SC_6H_5	H	O	(P)	(88-89°)

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

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

Table - II

Saligenin Cyclic Phosphoramidates and Phosphoramidothionates with Physical Properties.



Code No.	R_1	R_2	X	*Procedure	b.p. °C/mm Hg (m.p. °C)
K-19	CH_3	H	O	A	(87)
K-22	C_2H_5	H	O	A	(68)
K-41	n-C_3H_7	H	O	A	135-140/0.6
K-40	i-C_3H_7	H	O	A	(84)
K-42	n-C_4H_9	H	O	A	(46-47)
K-10	C_6H_5	H	O	A	(131-133)
K-20	CH_3	CH_3	O	A	(121)
K-23	C_2H_5	C_2H_5	O	B	133-6/0.5(44)
K-35	CH_3	H	S	A	120-123/0.2
K-37	C_2H_5	H	S	B	Undistilled liquid
K-36	CH_3	CH_3	S	B	118-122/0.2
K-38	C_2H_5	C_2H_5	S	B	110/0.2

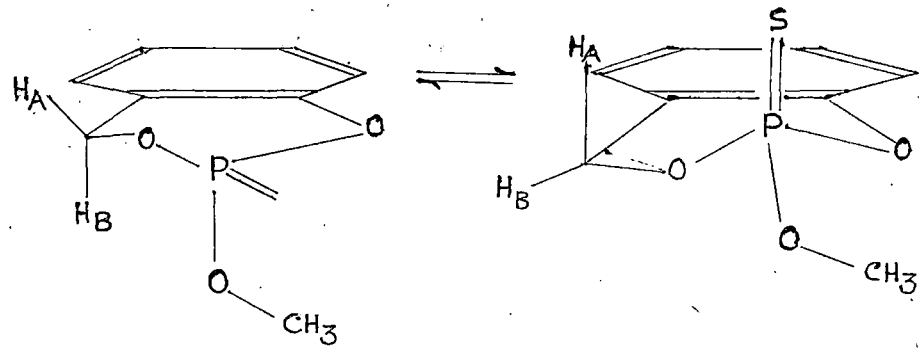
* Tertiary amine (A) or potassium carbonate (B) was used as dehydrogen chloride agent.

5. Chemical and Biological Properties of Salithion:

Here we concentrate our discussion to the important properties of salithion[5] relating to its structure, chemical and biological properties.

Pure salithion is a colourless crystalline powder: m.p. 55° - 56°C; practically insoluble in water but easily soluble in acetone and benzene, moderately soluble in cyclohexane, toluene and xylene; vapour pressure 1.5×10^{-6} mm Hg at 25°C; UV λ_{max} nm (ϵ) 274(860), 267(860). Salithion has characteristic IR band at 1020 cm^{-1} for P-O-CH_2 in the hetero ring. NMR $\delta(CS_2)$ ppm ; 3.76 (3H, doublet, J_{PH} = 14 Hz, CH_3), 5.21 (2H, doublet, J_{PH} = 15 Hz, CH_2), 6.8 - 7.2 (4H, multiplet, benzene ring). The signal at upper field of the doublet at 5.21 ppm slightly splits further (1.5 Hz). This becomes much significant at -30°C, suggesting the methylene protons (H_A , H_B) are not equivalent with each other, but the

dioxaphosphorin ring is conformationally mobile in a solution (Fig. 3)



(III) (IV)

Fig. 3 Conformational change of salithion hetero ring.

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

Salithion gives a characteristic fragmentation pattern in mass spectrometry (31). It gives an intense peak of $[M - CH_3]^+$ (m/e 201) by β-cleavage occuring at the exocyclic ester group. Another fragmentation process is the direct loss of SH followed by the elimination of formaldehyde (Fig. 4).

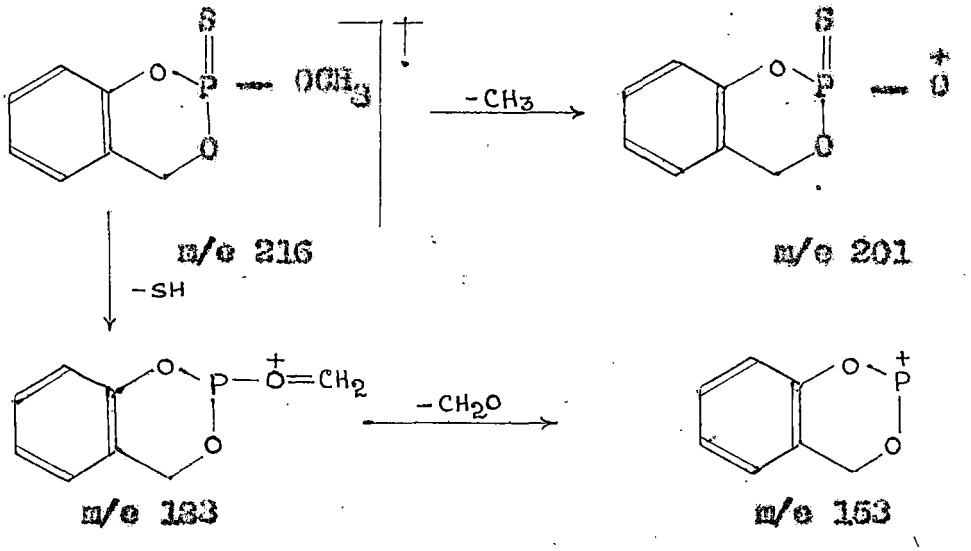


Fig. 4. Fragmentation of salithion in mass spectrometry.

Salithion is converted to its oxon analog (salioxon) by the action of bromine water. Since salioxon (2-methoxy-4H-1,3,2 benzodioxaphosphorin 2-oxide) is some thousands times more active in cholinesterase inhibition than salithion, an enzymatic method after the oxidation can be used for the residue analysis of salithion[32].

Salithion is converted into S-alkyl saligenin cyclic phosphorothiolates by heating with alkyl iodides (the Pistchimuke reaction)[33]. This reaction is accelerated in such polar compound 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)[33,34]. Saligenin is demethylated to form the salt of saligenin cyclic phosphorothionic acid by the action of certain neucleophiles such as cyclohexylamine[5] and potassium dimethyldithiocarbonate[5,35]. The later reagent is particularly suitable for the preparation of MTBO by methylating the obtained salt with methyl iodide. MTBO is a unique phosphorylating agent[5]. The reactions of salithion are summarized in Fig. 5.

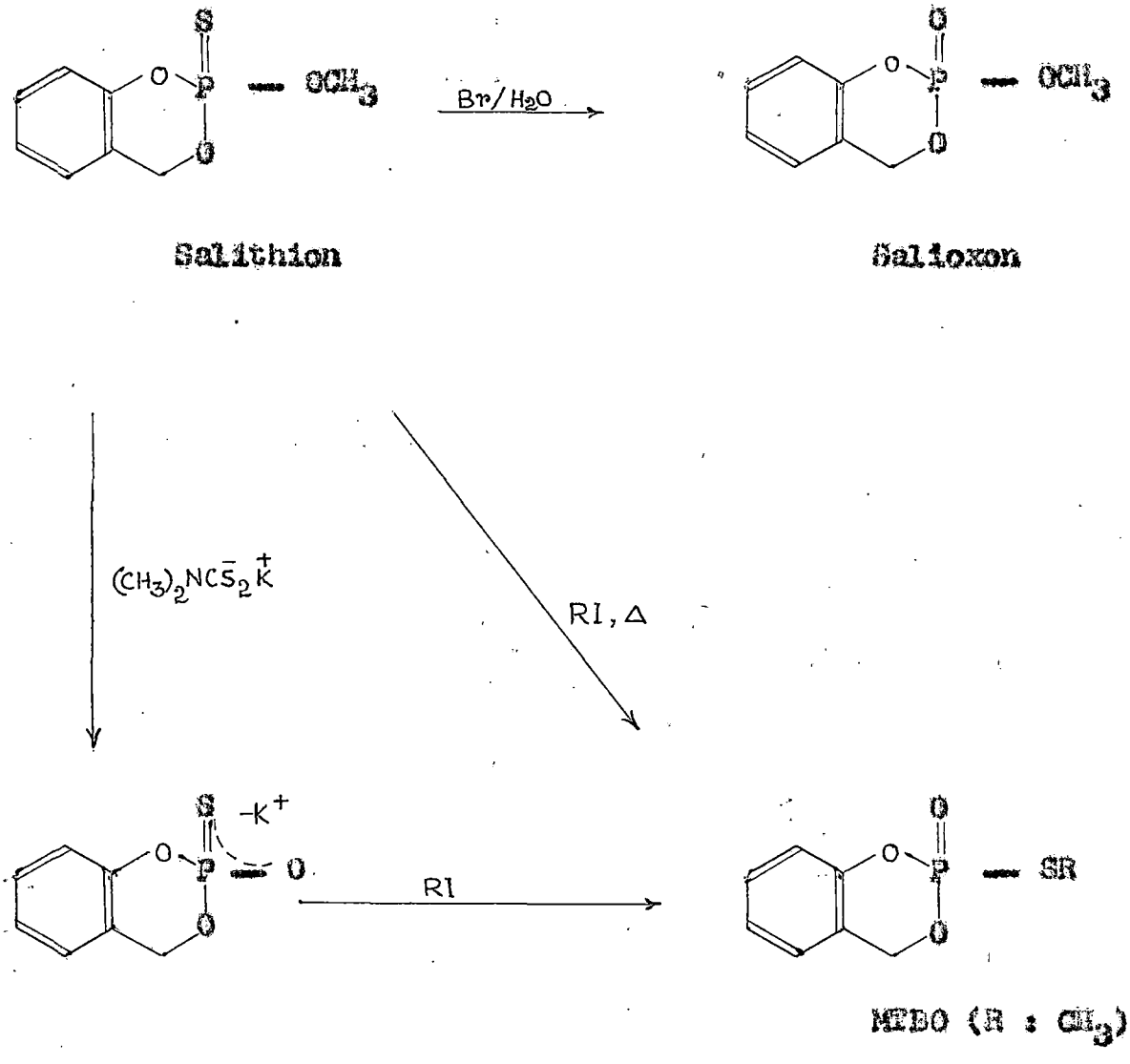


Fig. 5 - Reaction of Salithion.

Salithion is relatively unstable in storage. Some secondary amines, such as carbazole and N-phenyl α-naphthylamine, stabilized the formulation[36]. In a phosphate buffer (pH 7.7), salithion is hydrolysed slowly through opening of the hetero ring

by the P-O (aryl) bond cleavage[5], the hydrolysis rate constant (25°) K = 2.4 x 10^{-4} min^{-1}. The rates of hydrolysis of the corresponding cyclic methylphosphonate, S-methyl phosphorothiolate (the thiolate isomer of salithion, MTBO), methyl phosphate (salioxon), and N-methyl phosphoramidate are, respectively 30, 60, 6 and 0.6 times greater than that of salithion. Salithion is completely hydrolyzed by heating at 100°C for 5 minutes with N/6 sodium hydroxide to yield saligenin. The hydrolysis of salithion is shown in Fig. 6.

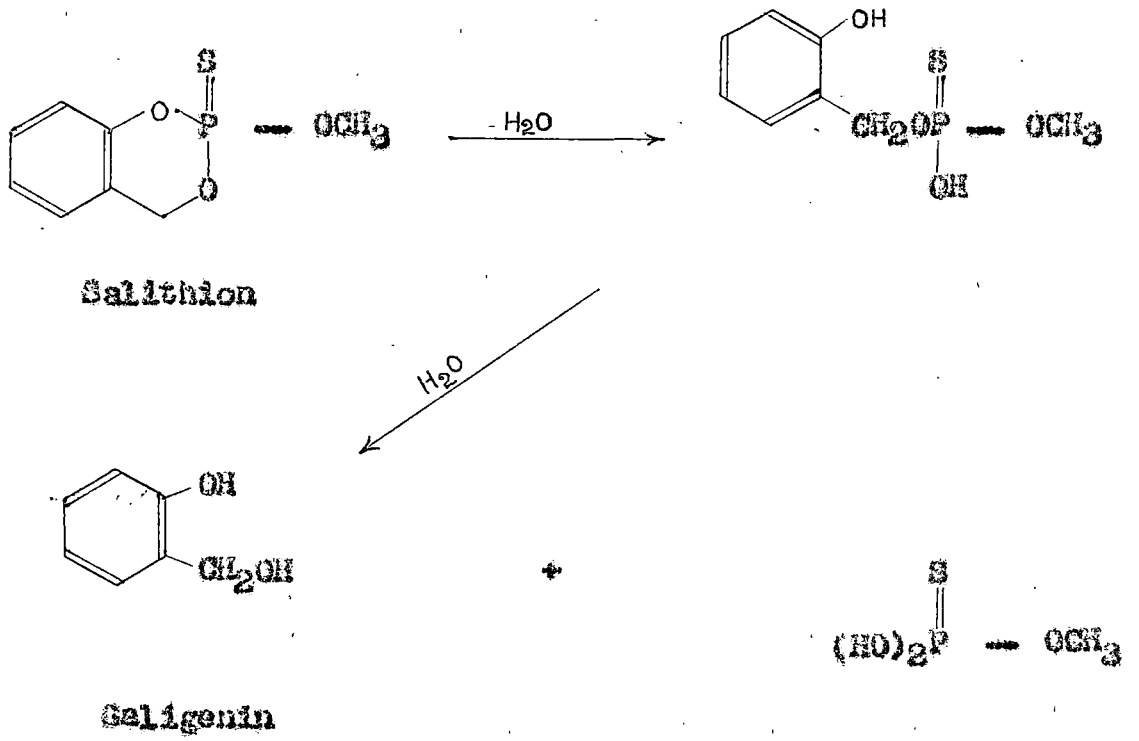


Fig. 6. Hydrolysis of Salithion.

Salithion is a wide-spectrum insecticide for use in orchards and vegetable gardens. It is particularly effective to control lepidopteran larvae, melaybugs, aphids and mites. It manifests the insecticidal action not only as contact and stomach poisons but also as a fumigant[37].

Salithion ^{32}P applied topically to houseflies rapidly absorbs into the body (42% after one hour). The major part was degraded in the body and about 4% of applied or 10% of absorbed salithion remains as salithion and salioxon for 24 hours. On the other hand, salithion ^{32}P administered orally to mice was rapidly degraded and excreted. After one hour, 78% of administered salithion was hydrolysed in the body. After 3 hour, 58.7% excreted and only 2.4% remained in the body[38].

Mihara et al[39] investigated the metabolism of salithion in rats and plants using ^{14}C-labelled compound. When rats were treated orally with salithion-4-^{14}C at the dosage of 9 mg/kg, 72, 82 and 91% of the radioactivity were excreted respectively after 12, 24 and 48 hours. The radionutograms of whole body showed a trace of radioactivity remained only in liver, kidney and lung after 24 hours. The radiocarbon was completely excreted during one week. This was also the case, even though the dosage was increased or repeated several times. No radioactive carbon dioxide was expired.

The metabolic pathways of salithion in rats and plants have been studied [1]. These are summarized [5] in Fig. 7.

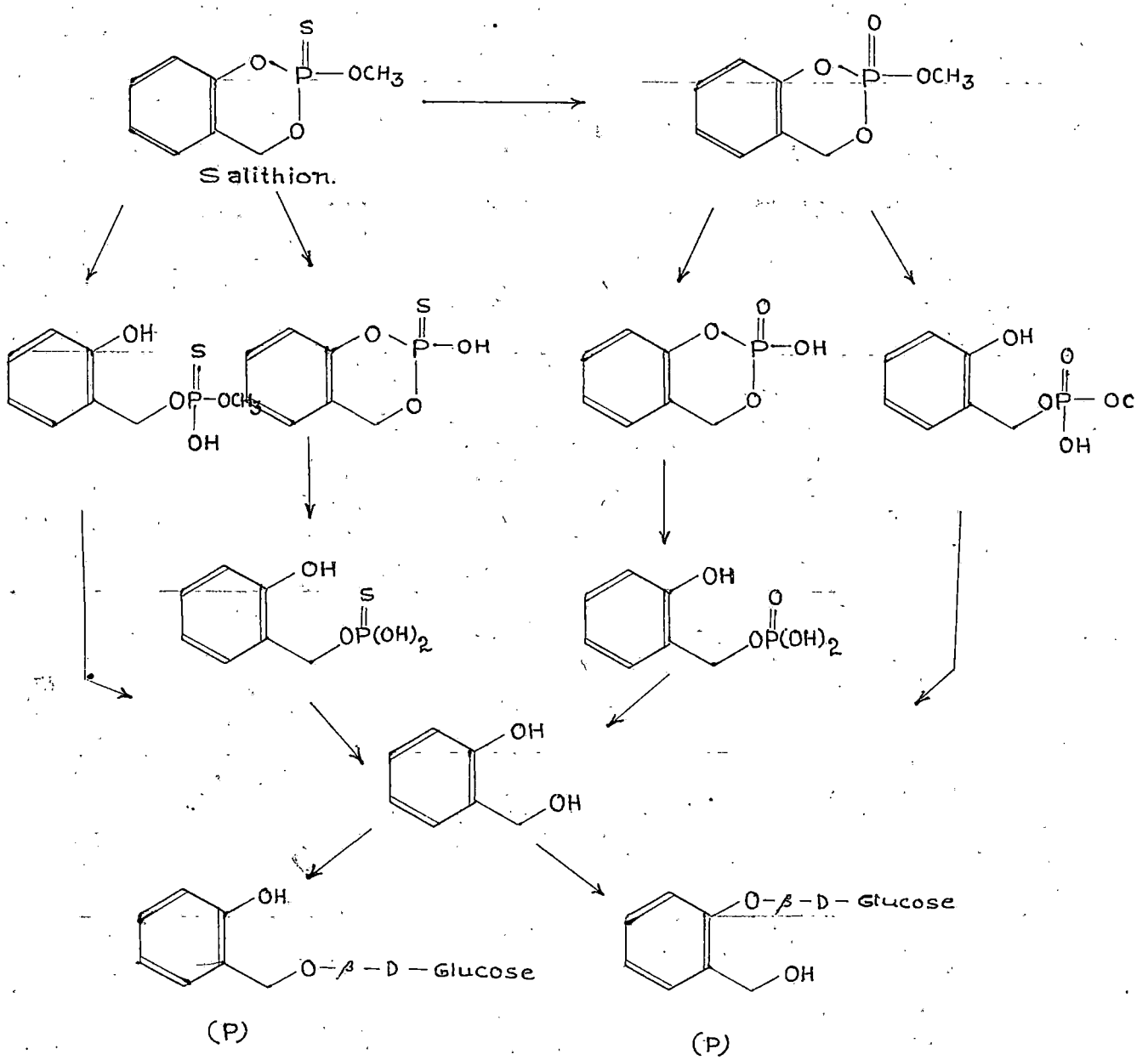


Fig. 7. Metabolic pathways of salithion in rats and plants (P-denotes the metabolite found in plant only).

It was shown that the biodegradation of salithion and salioxon proceeded through demethylation and ring-opening by P-O-aryl-bond cleavage. The metabolic pathways in plants only differed from those in glycoside conjugation of saligenin. About 10% of salithion absorbed was found in the bean plant whose roots had been soaked in the nutrient solution 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 the leaves was vaporised. Vaporization of salithion from a nutrient solution was also observed. This causes a fumigant action to kill insects on the plants.

The acute toxicity study of salithion for mammals was performed by Oshima[37]. The oral LD_{50} for mice is 91.3 mg/kg, for male rat 82-125 mg/kg, for female rats 102-180 mg/kg. The subcutaneous LD_{50} for male rat is 142 mg/kg and for female rats is 152 mg/kg. The acute oral LD_{50} for hen is 110 mg/kg.

Studies on the chronic toxicity of salithion was performed[5]. Rats fed for 24 months with 10 ppm salithion showed slight decrease in cholinesterase activities. No effect was observed in the rats fed with 3 ppm salithion. No histological lesion was found in any organs of rats fed with 100 ppm. In men and women administered orally 0.02 mg/kg/day of salithion for 21 days followed by 0.05 mg/kg/day for 14 days, no effect was found in

the activity of erythrocyte acetylcholinesterase. No effect was observed in fertility of rats for three generations fed with 10 ppm salithion. Carsinogenicity was not observed.

6. Chemical Hydrolysis of Saligenin Cyclic Phosphorus Esters.

The catalytic hydrolysis of saligenin cyclic phosphorus esters by phosphate ion has been reported[28b]. The chemical reactivity of the cyclic phosphates with nucleophilic agents should be influenced by the electronic character of the substituent. The relative reaction rate may be theoretically predicted. The reaction rate for the following derivatives follows the order : butoxy < propoxy < ethoxy < methoxy < phenoxy < phenyl. Again in another series, the relative reaction rate is in the order NR_2 < NHR < OR < R. These have been supported experimentally[40,42,28a].

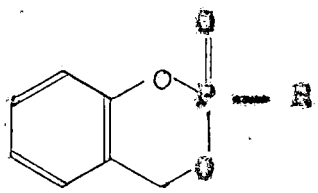
Saligenin cyclic phosphates were hydrolysed in pH 7.7 phosphate buffer to give O-hydroxy benzyl hydrogen phosphonates which gave chloroform-insoluble dyes with aminoantipyrine[6]. This is a characteristic reaction of cyclic phosphorus ester of saligenin. The first order hydrolysis constents (K_{hyd}) are given in Table-III. Phosphonic acid esters are generally more unstable than phosphoric acid esters. The rate constants of alkyl phosphonates are about 10 times larger than those of corresponding phosphates. There is a smaller difference between aromatic derivatives. Ethyl phosphonate is much stable than methyl

phosphonate. The presence of unsaturation or chlorine makes the esters unstable.

It is interesting that, in the series of alkyl derivatives there is a relationship between rate constant and insecticidal activity (LD_{50}). [Table - III and Table - IV]. The higher the reactivity is, the stronger the insecticidal activity. However, the aryl derivatives do not follow this relationship; they are more reactive than alkyl derivatives, but are almost non-insecticidal. The size of the substituent appears to be more important for biological activity than the electronic property of the substituent.

Table - III

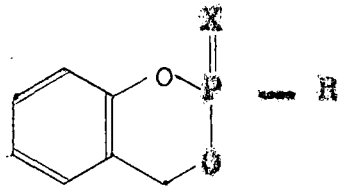
Hydrolysis rate constants of some saligenin cyclic phosphorus esters in phosphate buffer (pH 7.7) at 25°C.



R	K_{hyd} min^{-1}	R	K_{hyd} min^{-1}
CH_3	2.22×10^{-2}	OCH_3	1.42×10^{-3}
C_2H_5	4.25×10^{-3}	OC_2H_5	5.04×10^{-4}
$CH = CH_2$	1.39×10^{-2}	-	-
CH_2Cl	2.00×10^{-1}	-	-
CH_2CH_2Cl	1.41×10^{-2}	OCH_2CH_2Cl	2.58×10^{-3}
C_6H_5	1.28×10^{-2}	OC_6H_5	6.30×10^{-3}
NHC_6H_5	2.40×10^{-4}	$OC_3H_7(n)$	3.79×10^{-4}
$NHCH_3$	1.54×10^{-4}	$OC_4H_9(n)$	3.29×10^{-4}
$N(CH_3)_2$	Negligibly small.		

Table-IV

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



X	R	K_{hyd} min^{-1}	LD_{50} (μ g/female housefly)
O	CH_3	2.22×10^{-2}	0.19
O	C_2H_5	4.25×10^{-3}	0.17
O	i-C_3H_7	-	0.33
O	Sec-C_4H_9	-	7.0
O	t-C_4H_9	-	>10(0%)
O	$CH : CH_2$	1.39×10^{-2}	0.68
O	CH_2Cl	2×10^{-1}	<10 (60%)
O	CH_2CH_2Cl	1.41×10^{-2}	>0.99
O	C_6H_5	1.28×10^{-2}	>10 (10%)
O	OCH_3	1.42×10^{-3}	0.035
O	OC_2H_5	5.04×10^{-3}	0.33

Contd.....

Table-IV (Contd.....)

X	R	K_{hyd} min^{-1}	LD_{50} (μg/female housefly)
O	O-n-C_3H_7	3.79×10^{-4}	7.1
O	O-n-C_4H_9	3.29×10^{-4}	10 (40%)
O	O-CH_2CH_2Cl	2.58×10^{-3}	0.49
O	O-C_6H_5	6.30×10^{-3}	10 (3)
O	$NHCH_3$	1.54×10^{-4}	0.05
O	$N(CH_3)_2$	Negligibly small	0.40
O	NHC_6H_5	2.40×10^{-4}	10 (5%)

7. Biological Activities and Structural Relationship.

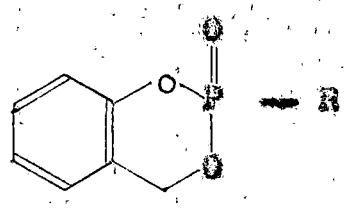
The soligenin cyclic phosphate esters have interesting biological activities. Some of them are neurotoxic, causing ataxia in higher animals. Others do not show such harmful activity but do have high insecticidal activity, systemic activity and fungicidal activity. Their biological activities include 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-V. All aryl soligenin cyclic phosphates manifest a high delayed neurotoxicity to cause ataxia in hens and high synergistic activity with

malathion (9,41). The arylphosphonate analogs showed similar biological activities but less in the neurotoxicity. On the other, the corresponding cyclic esters having a small alkyl group on phosphorus, i.e. 2-alkyl, 2-alkoxy-, and 2-alkylamino-4H-1,3,2-benzodioxaphosphorin 2-oxides, did not cause ataxia in hens with any sublethal doses and only weakly potentiated the toxicity of malathion (9). The interesting feature is that, the alkyl derivatives reveal high insecticidal activity, whereas, the aryl cyclic esters do not (8).

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 salicyloxyphosphinylenzymes (14,4) (VI)(Fig.8). This involves by opening of the cyclic ester structure at the P-O aryl bond. When the size of the exocyclic substituent R in (V) increases, the ester becomes a more selective inhibitor of aliesterase (42). Whereas, it becomes a more selective inhibitor of cholinesterase when the substituent is small. Thus the O-tolyl derivative (M), for example, is 130 times more selective to inhibit aliesterase than cholinesterase.

Table-V

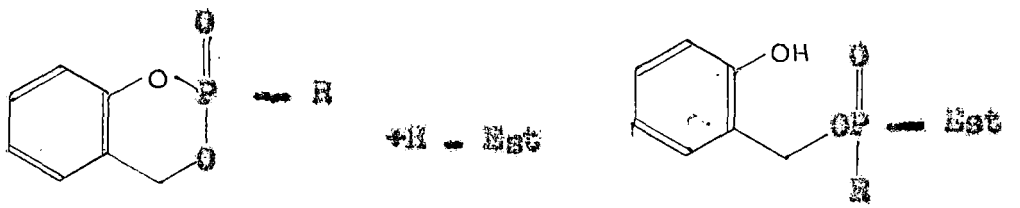
Effects of the exocyclic substituent (R) on biological activities of saligenin cyclic phosphate (V)



(V)

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

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



(V) (VI)

Fig. 8 Reaction of Saligenin cyclic phosphates with esterase (H-Est)

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

The heterocyclic structure of saligenin cyclic phosphorus esters is merely for the chemical reactivity of the phosphorus atom towards nucleophiles including the active site of esterase and is never requirement for the delayed neurotoxicity. As for example, although Tri-o-ethyl phenyl phosphate (TPEP) has the neurotoxicity[13], is unable to be transformed into a cyclic ester structure.

Johnson found "neurotoxic esterase" in nervous tissues which is specifically sensitive *in vivo* to neurotoxic organophosphorus esters[43]. The esterase is unlike acetylcholinesterase but similar to chymotrypsin and trypsin in the structure -activity relationship of inhibitors[44].

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 (Table VI - XIV):

Various series of cyclic esters of saligenin derived from pentavalent phosphorus acids have been examined for insecticidal activity. They include phosphates[19], phosphoramidates[23], phosphorothiolates[26], phosphonates[28] and their thiono analogs (Table VI - XIV). The insecticidal activity of the esters appears to relate with the size of the exocyclic substituent on the phosphorus atom (Table-VI).

The cyclic esters in any series having an aryl group as an exocyclic substituent on the phosphorus atom have either poor or no insecticidal activity. In all the series, methyl derivatives are much more active than higher alkyl derivatives, except for phosphonate series (Table - XI) in which ethyl

derivatives are more active than methyl derivatives. N, N-dialkyl phosphoramidates are much less active than mono-alkyl derivatives (Table-XIII). Thus saligenin cyclic methyl phosphate (Table-X), methyl phosphorothionate (Table-IX), N-methyl phosphoramidate, N-methyl phosphoramidothionate (Table-XIII), methyl phosphonothiolate and ethyl phosphonothiolate (Table-XI) 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 Å) 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°.

Furthermore, the introduction of any type of substituent at any position of the benzene ring and on the carbon atom of the hetero ring decreases the activity (29) (Table-VII). Thus, the simplest phosphorothionate, salithion, is the most effective insecticide in the whole series of saligenin cyclic phosphorus esters.

An outstanding contrast is observed in the effect of para-substitution between the salithion series and parathion series. Table (VIII) shows the effect of the electronic character of the substituent in the para-position of the phenolic ester group upon the insecticidal activity. Any substituents, either electron-withdrawing group or electron-releasing group decreases the

insecticidal activity. Table-VIII shows that the insecticidal activity of diethylphenyl phosphorothionates (II) is progressively increased by p-substitution of the phenyl ring in the increasing order of the electron withdrawing ability of the substituent, whereas neither electron withdrawing nor electron releasing group increases the activity of salithion (VIII). 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.

The systematic activity of some saligenin cyclic phosphoramidates against rice stem-borers and green rice leafhoppers on rice plants are observed (Table-XIII). Methyl phosphoramidate is more active than Schradan but less active than thimet against rice leafhoppers. Against rice stem-borers cyclic N-methyl phosphoramidothionate is superior to lindane and Diazinon. Salithion also shows more or less systemic activity against army worm and mite. No systemic activity has been observed in other compounds.

7.(b) Nematocidal Activity (Table XI and XIII, page-84 & 85):

Some saligenin phosphonothionates, phosphoramidates and phosphoramidothionates show nematocidal activity. Phosphoramidates and phosphoramidothionates are very effective to

kill nematodes (Table - XIII)[8]. N-methyl phosphoramidate is most active in the series of saligenin derivatives against the non-parasitic soil nematode Rhabditis suspended in water[23]. Owing to the instability in water, the cyclic phosphates and phosphonates are almost inactive, but their thiono analogs are effective against Rhabditis (Table XI, XIII).

Some saligenin cyclic aryl phosphonothionates are more effective as nematocides than the cyclic N-methyl phosphoramidate against the rice white tip nematodes, though they have very low insecticidal activity. Cyclic phenyl - and p -tolyl-phosphonothionates are the most effective nematocides in the series. These aryl phosphonothionates exhibit also a high activity against filaria in cotton rats (Litomosoides carinii)[5]. It is interesting to note that these aryl phosphonothionates are poor insecticides, whereas they are more potent to kill nematodes than salithion, suggesting the cholinesterase or other critical target of nematodes may differ in nature from the insect cholinesterase.

The correlation between the structure and the nematocidal activity of phosphoramides and phosphoramidothionates are similar to that in the insecticidal activity for housefly.

7.(c) Fungicidal Activity (Table XIV and XV, Page 88-89):

Salithion has no fungicidal activity. But some sali-genin cyclic phosphorothiolates have fungicidal activity (Table-

XIV). 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 Piricularia oryzae (45). The protective values against Piricularia oryzae of the cyclic phosphorothiolates and related compounds are shown in the Table (XIV & XV). The data of some commercial fungicides including an organophosphorus compound, Kinson (O-ethyl S, S diphenyl phosphorodithioate) are shown in the Table (XIV) 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 inactive 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 (45).

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.(d) Anti - SH Enzyme Activity (Table XV, Page - 89):

The saligenin cyclic phosphorothiolates have high activity to alkylate (salicylate) mercaptans and to inhibit "SH-enzymes" such as yeast alcohol dehydrogenase[31]. The activity seems to be related to fungicidal property but not with the insecticidal activity.

I_{50} values for alcohol dehydrogenase of some saligenin cyclic phosphorus ester are shown in (Table-XV). 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 high insecticidal property is almost inactive toward 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. 9.

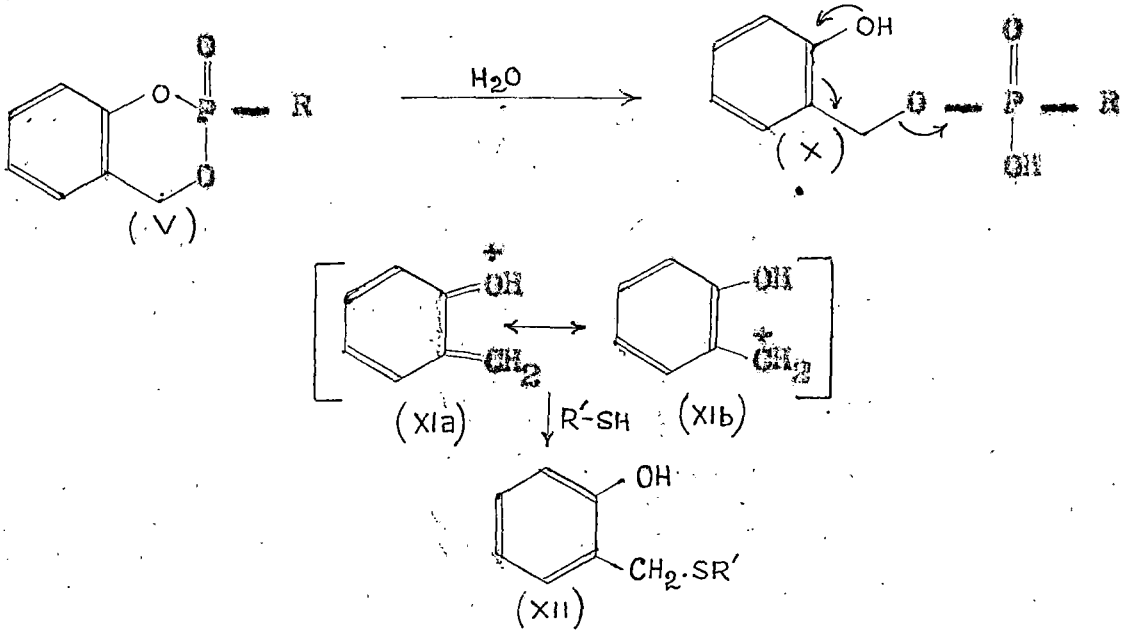


Fig. 9

Saligenin cyclic phosphorothiolates are partially hydrolysed by opening of the heterocyclic P-O-C-aryl bond, more easily than phosphate esters. In Fig. 9, the cyclic ester (V) is hydrolysed by the attack of hydroxide ion to yield salicyl phosphate (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[31].

It seems reasonable to conclude that the decrease of electron density on phosphorus atom causes the high reactivity of the phosphorothiolates. This is supported by the lower P = O frequency (1280 cm^{-1}) of the phosphorothiolates in comparison with that of the phosphates (1310 cm^{-1}).

Further investigation shows (Table-XV) 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.(e) Antiesterase Activity (Table-XVI, Page-90):

The relation of chemical structure to biological activities indicates that the specificity in biological activities of the saligenin cyclic phosphorus esters seem to be remarkably influenced by the steric characteristics of the exocyclic sybstituent on the phosphorus atom. This is evident when compared their specificities in enzyme inhibition (42).

The most insecticidal saligenin cyclic methyl phosphate (salioxon) is the strongest inhibitor of insect cholines-

terase. However, the highly neurotoxic aryl phosphate is a poor inhibitor of cholinesterase, but is a very specific inhibitor of aliesterase[1,42]. The less neurotoxic arylphosphonate occupies an intermediate position. In any series, when the size of the exocyclic substituent increases, the compound becomes a more selective inhibitor of aliesterase; in contrast, the compound carrying a small substituent is a more selective inhibitor of cholinesterase (Table-XVI). Aryl phosphonates are more specific inhibitors of pseudo-cholinesterase; alkyl phosphates are less specific and aryl phosphates are intermediate.

7.(f) Synergistic Activity (Table - XVII, XVIII and XIX Page 91-94):

Saligenin cyclic aryl phosphates and phosphonates have synergistic activity with malathion against insects and mites, particularly their resistant strain[46].

The joint action of the activity of some saligenin cyclic phosphorus esters with malathion has been examined by Eto et al[41] and compared with some phosphorus esters which are known as the synergists of malathion. Table-XVII shows that the aryl derivatives of saligenin cyclic esters are synergistic with malathion. They increase the toxicity of malathion 2.8 to 3.4 times at a 1:1 mixing ratio. The activities of them are more than propyl paraoxon 2-benzodioxaphosphorin-2-oxide, and 7-methyl-2-phenyl-4H-1,3, 2-benzodioxaphorin-2-oxide are the most synergistic against

susceptible housefly among the tested cyclic esters. Alkyl derivatives of saligenin cyclic phosphorus esters are much less synergistic or even antigonistic.

Synergism of saligenin cyclic phosphorus esters with malathion in a resistant strain of housefly has been tabulated in Table-XVIII. In this case, the synergistic effect of saligenin cyclic phosphorus esters has remarkably been increased. Even the alkyl derivatives act as synergist of malathion. Thus all of present cyclic esters are more active than the other tested organophosphorus synergists. Cyclic phenylphosphonate of methyl saligenin is the most effective synergistic against resistant housefly and increases the toxicity of malathion 14 times.

Synergistic effect on susceptible and resistant strains of green rice leaf hopper has been observed (41) and tabulated in Table (XIX). The toxicity of malathion to the susceptible insects becomes double by the addition of three saligenin cyclic phosphorus esters along with two other cyclic esters. Synergistic effect on resistant leaf hopper also increases by saligenin cyclic phosphorus esters but the values are lower than in the resistant housefly. They enhanced the toxicity of malathion 2.2 to 3.8 times.

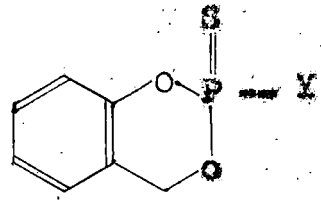
The synergism appears to result primarily from the inhibition of detoxication of malathion. Phosphate action

or esterase action can detoxify the toxicity of malathion. It has been observed for a great number of organophosphorus compounds that the synergism of malathion in mice and the degree of inhibition of ali-esterase *in vivo* are generally related[9]. 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*[42] have shown that aryl derivatives of saligenin cyclic phosphorus esters are the selective inhibitors of ali-esterase, whereas small alkyl derivatives are not so selective to ali-esterase inhibition. This appears to be responsible for their difference in synergistic properties.

Table - VI

Relation of structure to insecticidal activity (LD_{50} μg/housefly) of (VII)



(VII)

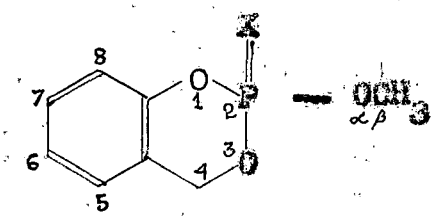
X / R	R		OR		SR		NHR	
	O	S	O	S	O	S	O	S
CH_3	0.13	0.31	0.04	0.05	0.09	0.13	0.05	0.04
C_2H_5	0.17	0.08	0.33	0.03	0.23	0.9	0.66	0.48
n-C_3H_7	0.33[a]	0.09[a]	7.1	-	2.34	2.2	1.50	-
n-C_4H_9	7.05[b]	-	(40)	-	6.30	10	(54)	-
C_6H_5	(0)	0.3	(3)	2.0	2.2	(0)	(5)	-

a = isopropyl, b = Sec.-butyl.

Figures in parentheses are percentage mortality at 10 μg/fly.

Table - VII

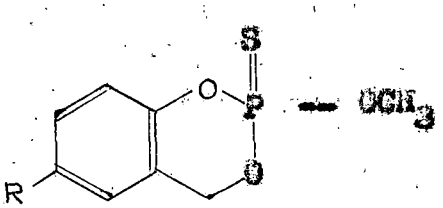
Effect of substituent (R) on insecticidal activity (LD_{50}/µg/house-fly).



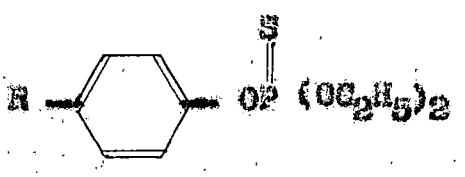
R	X	
	S	O
H	0.06 (Salithion)	0.035 (Salioxon)
4-CH_3	-	3.35
6-CH_3	2.00	0.1
7-CH_3	0.23	0.43
8-CH_3	1.30	2.0
6-Cl	1.75	0.09
8-Cl	0.13	0.23
β -CH_3	0.30	0.33
β -CH_3OCH_2	3.55	0.99
β -Cl	-	2.07

Table - VIII

Effect of P-substitution on insecticidal activity of Salithion (VIII) and parathion (IX) series.



(VIII)



(IX)

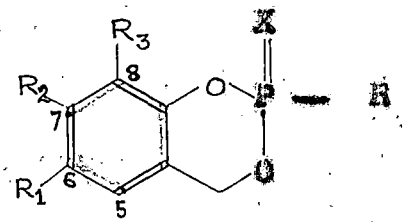
R	σ[a]	Relative insecticidal activity[b]	
		(VIII)	(IX)
OCH_3	-0.268	9.2	0.1
CH_3	-0.170	2.6	0.1
H	0.000	100.0	0.1
C_6H_5	+0.009	12.8	-
Cl	+0.226	3.0	0.33
$COCH_3$	+0.87	2.0	2.5
NO_2	+1.27	1.7	100.0

a = Hammett's substituent constant

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

Table - IX

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	0.6
S	C_6H_5	H	H	O-n-C_3H_7	1.0

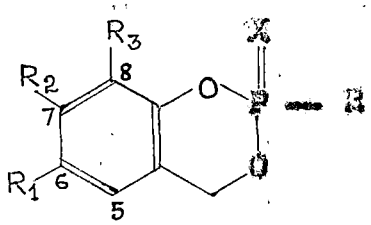
Contd.....

Table - XX (Contd.....)

X	R_1	R_2	R_3	R	LD_{50} (μ g/housefly)
S	OCH_3	H	H	OCH_3	0.55
S	$COCH_3$	H	H	OCH_3	2.6
S	Cl	H	H	OCH_3	1.75
S	Cl	H	H	$NHCH_3$	0.06
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 - I

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
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

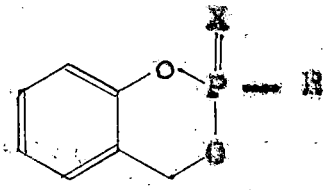
Contd.....

Table - X (Contd.....)

X	R_1	R_2	R_3	R	LD_{50} (μg/housefly)
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 - XI

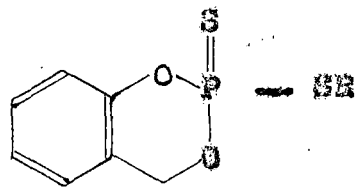
Saligenin cyclic phosphonates and phosphonothionates : toxicity, insecticidal and nematocidal activity.



X	R	LD_{50} µg/fly (Housefly)	LD_{50} mg/kg (House oral)	LD_{100} ppm (nematodes-Rhabditis)	
O	CH_3	0.19	25-50		
O	C_2H_5	0.17	25-50		
O	i-C_3H_7	0.33	-		
O	Sec-C_4H_9	7.0	-		
O	t-C_4H_9	>10(0%)	-		
O	$CH = CH_2$	0.68	>100		
O	CH_2Cl	<10(60%)	25-50		
O	CH_2CH_2Cl	0.99	50-75		
O	C_6H_5	>10(0%)	-		
S	CH_3	0.31	5-10		25
S	C_2H_5	0.08	5-10		25
S	i-C_3H_7	0.09	-		-
S	CH_2Cl	1.14	25-50		25
S	C_6H_5	0.30	-		-

Table - XII

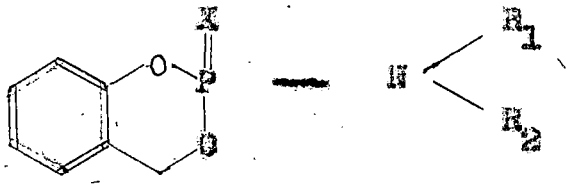
Insecticidal activity of Saligenin Cyclic Phosphoro-dithionates.



R	LD_{50} (µg/fly)
CH_3	0.18
C_2H_5	9
n-C_3H_7	2.2
i-C_3H_7	5
$C_3H_5(CH_2-CH = CH_2)$	1.7
n-C_4H_9	10
C_6H_5	> 10(0%)

Table - XIII

Saligenin cyclic phosphoramidates and phosphoramidothionates : Insecticidal activity, toxicity and nematocidal activity:



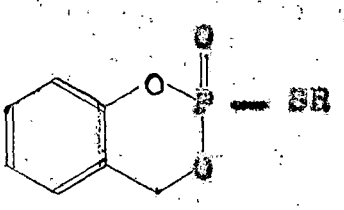
X	$N\begin{matrix}R_1\\R_2\end{matrix}$	LD_{50} (μg/female House fly)	LD_{50} (μg/mg Rice stemborer)	LD_{50} μg/gm (Green rice leaf-hopper)	LD_{50} mg/kg (House)	Minimum conc. (ppm) to kill 100% nematodes-Rhabditis
O	NHMe	0.05	2.84	0.04	6-7.5	< 10
O	NHEt	0.60	22.20	3.50	30-50	10-25
O	NH-n-Pr	1.50	33.60	33.0	> 50	25-50
O	NH-i-Pr	3.44	103.34	> 350	> 50	50-100
O	NH-n-Bu	< 10(54%)	> 214	> 400	> 50	25-50
O	NH-Ph	> 10(6%)	-	-	-	-
O	$N(Me)_2$	0.3	13.80	4.0	-	> 200(10%)

Table - XIII

X	$N\begin{matrix}R_1\\R_2\end{matrix}$	LD_{50} (µg/female House fly)	LD_{50} µg/gm (Rice stem-borer)	LD_{50} µg/gm (Green rice leaf-hopper)	LD_{50} mg/kg (Mouse)	Minimum conc. (ppm) to kill 100% nemato-des-Rhabditis
O	$N(Me)_2$	0.3	13.80	4.0	-	200 (10%)
O	$N(Et)_2$	>10(0%)	167.61	34.10	>50	100-150
S	NHMe	0.044	4.84	4.1	20-30	25-50
S	NH.Et	0.48	36.25	-	-	-
S	$N(Me)_2$	0.33	-	-	-	50-100
S	$N(Et)_2$	0.63	-	-	-	>200(30%)
S	OMe (Salithion)	0.05	1.13	30.6	88	-
O	OMe (Sallioxon)	0.035	2.16	1.8	52	-
Parathion		0.040	3.43	3.6	5-7	-
Malathion		0.060	-	0.8	347	-
D-D mixture (mixture of 1,3-dichloropropane and 1,2-dichloropropane)		-	-	-	-	>800(85%)

Table - XIV

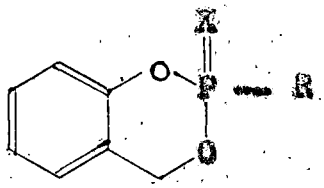
Insecticidal and fungicidal activity of saligenin cyclic phosphorothiolates:



R	LD_{50} μg/gm (oriental housefly)	Protective value % against Piricularia oryzae				Therapeutic value(%) to P.oryzae at 200 ppm
		200 ppm	100 ppm	50 ppm	25 ppm	
CH_3(MEBO)	3.00	100	100	100	84.8	7.1
C_2H_5	11.21	100	93.7	92.5	61.6	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 600 ppm)	-	-	-	-
Hinosan	-	100	-	86.2	-	95.2 (at 250 ppm)
Blasticidins	-	-	-	88.3	-	97.6
Pentachlorobenzyl alcohol	-	98.8	98.8	93.5	-	0

Table - XV

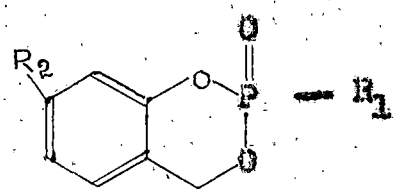
Chemical, Biological and Anti-Fungal activities of some saligenin cyclic phosphates and their thio analogs.



X	R	Hydrolysis %	Cysteine reacted %	I_{50} yeast alcohol dehydrogenase ($M \times 10^{-6}$)	Protective value against *Piricularia oryzae* % 50 ppm	600 ppm
O	SCH_3	66	55	4.5	100	-
O	SC_2H_5	81	60	4.4	93	-
O	OC_6H_5	55	45	6.8	-	-
O	OCH_3	17	10	62	-	65
S	OCH_3	6	5	100	-	52

Table - XVI

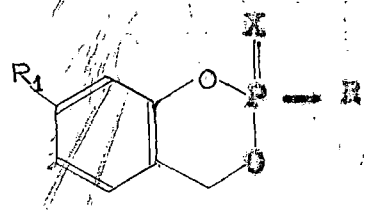
The Inhibition of Housefly, Human Blood and Horse Serum-Esterases by some Saligenin Cyclic Phosphorus Compounds.



R_1	R_2	Housefly $I_{50} \times 10^8$(M)		Human blood $I_{50} \times 10^8$(M)		Horse plasma $I_{50} \times 10^8$(M)	
		ChE	AliE	P-ChE	t-ChE	AliE	Malathio-nase
OCH_3	H (Saliloxon)	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	-

Table - XVII

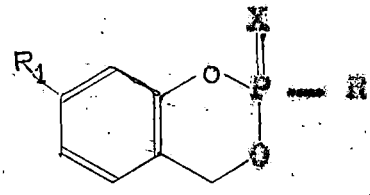
Joint action of some saligenin cyclic phosphorus esters and some other phosphoric esters with malathion against susceptible house flies.



X	R_1	R	LD_{50} µg/fly alone	LD_{50} µg/fly with malathion 1:1	Cotoxicity co-efficient.
Malathion			0.46		
O	H	OMe	0.02	0.06	0.6
O	H	OEt	0.16	0.14	1.7
S	H	OEt	0.11	0.22	0.8
O	H	OPh	c.10(30%)	0.38	2.3
O	H	O-o-Tol	c.10(40%)	0.26	3.4
O	H	Ph	c.10(60%)	0.36	2.5
O	Me	Ph	c.10(70%)	0.26	3.4
TOCP			>10(0%)	0.90	1.0
Dibrom			0.03	0.008	7.1
Propyl paraxon			0.10	0.11	1.5
Isopropyl paraoxon			0.38	0.06	4.9

Table - XVIII

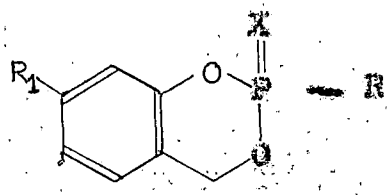
Synergistic effect of saligenin cyclic phosphorus esters with malathion against resistant house flies and comparison with other phosphate synergists.



X	R_1	R	LD_{50} µg/fly alone	LD_{50} µg/fly with malathion 1:1	Cotoxicity Co-efficient
Malathion			2.54		
O	H	OMe	0.15	0.06	4.7
O	H	OEt	0.40	0.22	3.1
S	H	OEt	0.20	0.10	3.6
O	H	OPh	>10(10%)	0.55	9.2
O	H	O-o-Tol	>10(10%)	0.65	7.8
O	H	Ph	>10(25%)	0.57	8.0
O	Me	Ph	c.10(40%)	0.29	14.0
TOCP			>10(10%)	3.44	1.5
Dibrom			0.07	0.07	2.0
Propyl paraoxon			0.73	0.46	2.5
Isopropyl paraoxon			0.43	0.36	2.2

Table- XIX

Synergistic effect of saligenin cyclic phosphorus esters with malathion against susceptible and resistant green rice leafhopper.



X	R_1	R	LD_{50} (μg/susceptible g.r. leaf-hopper)		Cotoxicity Co-efficient	LD_{50} (μg/resistant g.r. leaf-hopper)		Cotoxicity Co-efficient
			alone	with malathion 1:1		alone	with malathion 1:1	
Malathion			0.003			0.021		
O	H	OMe	0.008	0.004	1.1	0.010	0.006	2.3
O	H	OEt	0.069	0.003	1.9	0.066	0.010	3.2
S	H	OEt	1.892	0.005	1.2	>2	0.019	2.2
O	H	OPh	0.240	0.003	2.0			
O	H	O-o-Tol	0.464	0.008	0.8	(0.0167)	0.015	2.2
O	H	Ph	0.218	0.005	1.2			

Table - XIX (Contd.....)

X	R_1	R	LD_{50} (µg/susceptible g.r. leaf-hopper)		Cotoxicity Co-efficient	LD_{50} (µg/resistant g.r. leaf-hopper).		Cotoxicity Co-efficient.
			alone	with malathion 1:1		alone	with malathion 1:1	
O	Me	Ph	3.120	0.003	2.0	>3	0.011	3.8
TOCP			>10	0.006	1.2	>(10)	(0.083)	1.1
Dibrom			0.208	0.005	1.2	0.533	(0.069)	1.3
Propyl paraoxon			0.006	0.002	2.0	-	-	-
Isopropyl paraoxon			0.211	0.003	2.0	-	-	-

R E F E R E N C E S.

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P A R T - III

P A R T - III

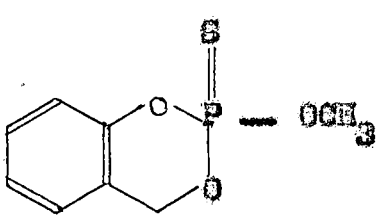
STUDIES ON SOME SALIGENIN CYCLIC PHOSPHORAMIDOTHIONATES HAVING FUNGICIDAL ACTIVITIES.

[Synthesis, fungicidal activity, insecticidal activity, acute oral toxicity, phytotoxicity, anticholinesterase activity, and chemical hydrolysis of some 6-nitro-saligenin cyclic phosphoramidothionates.]

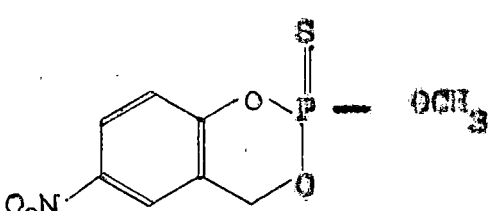
PART - III

AIMS AND OBJECTS OF THE PRESENT INVESTIGATION.

As stated previously (in Part - II) the cyclic organophosphorus esters of saligenin were discovered as the biologically active metabolites 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 commercialised as an insecticide. The introduction of any type of substituent at any position of the benzene ring decreases the insecticidal activity[1]. It has been reported[1] that the BD-8 (2 - methoxy - 6 - nitro - 4H - 1,3,2 - benzodioxaphosphorin - 2 - sulphide) is obtained as a paste in the reaction of O - methyldichloridophosphorothionate with 5 - nitro saligenin after purification through silicic acid column chromatography; and, this methoxy compound (BD-8) has about sixty times less insecticidal activity compared to salithion[1].



Salithion

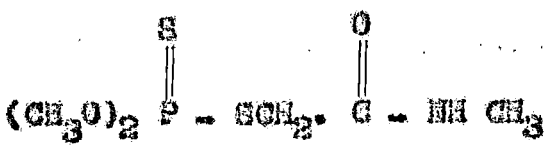


BD-8 (m.p. - 84°C)

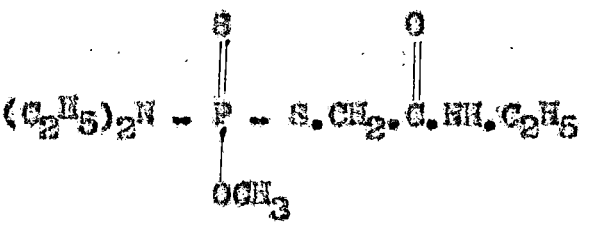
However, it has been observed[2] in this laboratory that the methoxy compound (BD-8) is a solid (m.p. 84°C), and has about 1.5 to 2 times greater oral insecticidal activity to cockroaches, Periplaneta americana (Linn) compared to salithion; and also its (BD - 8's) contact

insecticidal activity against grasshoppers, Oxya nitidula is comparable with that of malithion[3]. Our investigations on 6 - nitro saligenin cyclic phosphorus compounds have started with the finding of insecticidal activity in the compound BR-8.

Introduction of an amide group in place of an alkyl ester group often affords organophosphorus esters with fungicidal, herbicidal, and some-times insecticidal activity[4]. Phosphoramidates having a bulky alkyl group as iso-propyl, secondary - butyl, and cyclohexyl on the amido nitrogen atom show high herbicidal activity, whereas N - ethyl, N - methyl or N - monosubstituted phosphoramidate are suitable as insecticide. Although the insecticide dimethoate [dimethyl S - (N - methylcarbamoyl methyl) phosphorothiolothionate] has no fungicidal activity, its dialkylphosphoramidate analogs such as compound - I, show some fungicidal as well as acaricidal activity[5].



Dimethoate



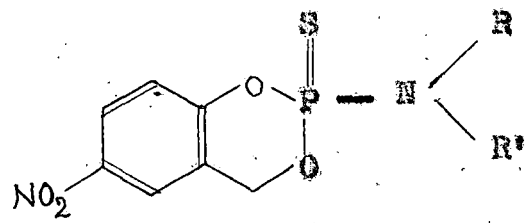
Compound - I

The S - alkylcarbamoylmethyl group is not required for the fungicidal effect, and some S - phenylphosphoramidothiolothionates are more active than compound - I. Phosbutyl (ethyl S - phenyl N - butylphosphoramidothiolothionate) is a practically useful fungicide in this series[6]. An amido analog of Kitazin, S - benzyl ethyl N - isopropylphosphoramido-

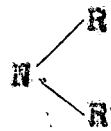
thiolate) was patented as a fungicide[7].

There are several other examples in literature from which it can be found that some phosphoramidothionates, phosphoramidothiolothionates, phosphoramides or phosphonamides in which the phosphorus atom is attached directly to the nitrogen atom of an amine or a heterocyclic compound such as phthalimide, imidazole or triazole have very good fungicidal activity[4,6,8,9].

These observations prompted us to undertake a systematic work on some 6 - nitro saligenin cyclic alkylamidophosphorothionates having the general structure (A),



(A)

where,  is cyclo-hexylamido, morphilino, dimethylamido, diethylamido, pyrrolidino, piperidino, isopropylamido, nonylamido or heptadecylamido. The work embodied in this dissertation is related to the investigation of the above mentioned compounds with reference to their chemical, fungicidal, insecticidal, toxicological, anticholinesterase, phytotoxic and hydrolytic properties besides structural elucidations by spectroscopic methods.

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A : MATERIALS AND METHODS

A. MATERIALS AND METHODS

1. PURIFICATION OF SOLVENTS AND OTHER CHEMICALS:

Throughout the preparative and other part of the work, the organic solvents and other chemicals used were purified and dried according to Vogel[1]. All other chemicals and solvents occasionally used during the work were of standard commercial products of high quality (EM, BDH, SM and/or Fluka quality).

2. SPECTROSCOPIC METHODS:

Infrared spectra were obtained with Beckmann IR-20 spectrophotometer in nujol mull. PMR spectra were recorded on Varian Model A - 60, EM - 390 and FT - 80A instruments in $CDCl_3$ solvent using TMS as an internal reference. Mass spectra were taken on Varian Model EM - 600 and MS - 30 instruments.

3. PREPARATION OF THIOPHOSPHORYL CHLORIDE ($PSCl_3$):

Thiophosphoryl chloride was prepared according to Moeller et al[2]

4. PREPARATION OF 5 - NITRO - SALIGENIN (2 - HYDROXY - 5 - NITRO - BENZYL ALCOHOL):

The 2 - hydroxy - 5 - nitrobenzyl alcohol as one of the starting materials for the synthesis of nitro-saligenin cyclic alkylamido phosphorothionates was prepared in the following manner:

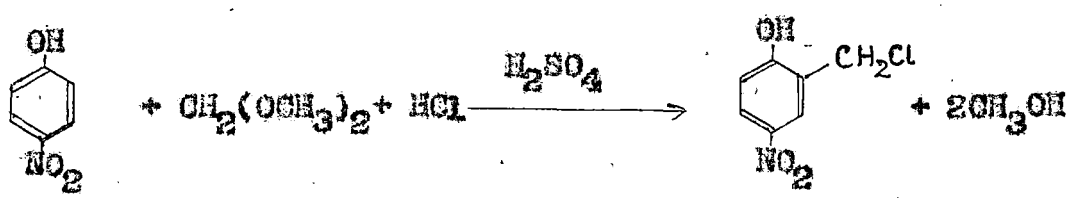
Preparation of the alcohol was done in two stages:

(i) Preparation of the 2 - hydroxy - 5 nitro benzyl chloride, and

(ii) the hydrolysis of the said chloride.

4.(1) PREPARATION OF 2-HYDROXY - 5 - NITROBENZYL CHLORIDE:

The 2 - hydroxy - 5 - nitrobenzyl chloride was prepared according to the method described in organic synthesis[3]. The reaction involved is :



The p - nitrophenol melting at above 112°C (Reidel, m.p. 114°C) was used for the preparation. The other ingradient methylal was synthesized afresh for every batch of preparation as follows:

760 ml. methanol was added to 400 gm anhydrous calcium chloride in a 3 lt. round bottomed flask equipped with a reflux condenser, 10.2 ml. conc. hydrochloric acid was added; then with cooling and constant stirring, 400 gm of 37 - 40% formaldehyde was slowly dropped in through a dropping funnel. It took about 2 hours for complete addition of formaldehyde (the reaction was strongly exothermic). When all the formaldehyde had been added, the mixture was heated for a few minute until the liquid boiled vigorously. The methylal came up

quickly on the upper layer, and after an overnight standing was fractionally distilled. The 42 - 45°C fraction was collected and stored in a tightly stoppered bottle in cold (freezer) before it was used.

In a one-litre, three-necked round bottomed flask equipped with a mechanical stirrer, a short reflux condenser, and a bent glass tube reaching sufficiently below, were placed 50 gm (0.36 mole) p - nitrophenol, 650 ml conc. HCl, 5 ml conc. H_2SO_4 and 76 gm (1 mole) methylal. The reaction mixture was stirred while the temperature was maintained at and around 70 ± 2°C for 4 - 5 hrs. by means of a water bath. During this time HCl gas was bubbled into the reaction mixture through the bent glass tube. The 2 - hydroxy- 5 - nitrobenzyl chloride began to separate as a solid after about one to one and half hour. At the end of the reaction, the mixture was cooled in a ice - bath for a period of 1-2 hour, more crystals separated. The solid materials, after filtration, were air dried for several hours to remove water; and then washed with benzene. The yield was about 40 - 45 gm. of a white product, m.p. 129 - 130°C.

4. (ii) HYDROLYSIS OF 2 - HYDROXY - 5 - NITROBENZYL CHLORIDE.

The said chloride was taken as suspension in water and heated slowly; the crystals would go into solution while the impurities form a deep brown oily layer at the bottom of the container. The mixture was then boiled gently to ensure complete hydrolysis. The solution was quickly filtered while hot. The light yellow crystals of 2- hydroxy - 5 - nitrobenzyl alcohol seperated as the filtrate got cooled. The product (m.p. 122 - 126°C) thus obtained was recrystallised from hot water; the

crystals were filtered, washed with cold dioxan: benzene (1:9) mixture, and dried in vacuum.

5. PREPARATION OF PHOSPHORAMIDOTHIOIC DICHLORIDES:

$$2\ RNH_2 + PSCl_3 \longrightarrow RNH.\ P(S)Cl_2 + RNH_2.HCl$$

(2mole) (1 mole)

or

$$RNH_2 + PSCl_3 \xrightarrow[1\ mole]{\text{Triethylamine}} RNH.\ P(S)\ Cl_2 + \text{triethylamine-hydrochloride}$$

(1 mole) (1 mole)

One mole $PSCl_3$ and two moles amine (or one mole amine and one mole triethylamine) were allowed to react at + 5° to - 5°C in benzene/chloroform as solvent. The amine solution was added dropwise very slowly with constant vigorous stirring. After an additional stirring period, the solid particles were filtered off the reaction mixture and the liquid phase was washed repeatedly with benzene/chloroform, subsequently with 2% cold hydrochloric acid, then with cold saturated solution of sodium chloride. The benzene/chloroform phase was then dried with anhydrous sodium sulphate and filtered; evaporation in Vacuo gave the desired phosphoramidothioic dichloride; this dichloride was used as such for the subsequent reaction.

The different phosphoramidothioic dichlorides were prepared as follows:

5.(i) CYCLOHEXYLAMIDOPHOSPHORODICHLORIDOTHIONATE:

Thiophosphoryl chloride (16.9 gm; 0.1 mole) in 100 ml benzene was allowed to react with cyclohexylamine (19.8 gm; 0.2 mole) in 20 ml benzene at -2° to 5°C; the amine solution was added dropwise very slowly with constant vigorous stirring. After an additional stirring period of two hours, the cyclohexylamine hydrochloride was filtered off, and the solution was washed with 2% cold hydrochloric acid saturated with sodium chloride, and then with cold saturated solution of sodium chloride. The benzene phase was then lyophilised with anhydrous sodium sulphate and filtered; evaporation in Vacuo gave the cyclohexylamidophosphorodichloridothionate. 16 gm (70% yield) white crystalline solid (m.p. 68 - 69°C) was thus obtained.

5.(ii) MORPHOLINOPHOSPHORODICHLORIDOTHIONATE:

A solution of morpholine (8.7 gm; 0.1 mole) in 20 ml benzene was added dropwise to a stirred solution of thiophosphoryl chloride (8.45 gm; 0.05 mole) in 50 ml benzene at -5° to + 5°C. The mixture was stirred at 5°C for 3 hr. and at room temperature for 16 hr. The mixture was worked up as in 5.(i). A clear viscous liquid which solidified after standing several days was obtained (7.0 gm; 64% yield,) m.p. 30 -31°C,:

5.(iii) N.N - DIETHYLAMIDOPHOSPHORODICHLORIDOTHIONATE:

Diethylamine was extracted in benzene from its aqueous solution by partition method, and its concentration was determined by volumetric method; before estimation of the amine the benzene phase

was dried with anhydrous sodium sulphate.

7.3 gm (0.1 mole, 50.12 ml. benzene extract solution conc. 0.1456 gm/ml) diethylamine was allowed to react with thiophosphoryl chloride (8.45 gm, 0.05 mole) in 50 ml. benzene at -5° to + 5°C and the mixture was worked up as in 5 (i). 8.0 gm (78% yield liquid possesing camphor - like odour was thus obtained.

5. (iv) N,N - DIMETHYLAMIDOPHOSPHORODICHLORIDOTHIONATE:

N, N - Dimethylamine was extracted in chloroform from its aqueous solution by partition method and its concentration was determined by volumetric method; before estimation of the amine, the chloroform phase was dried with anhydrous sodium sulphate.

Thiophosphoryl chloride (8.45 gm; 0.05 mole) in 50 ml chloroform was allowed to react with 4.5 gm (0.1 mole, 37.8 ml chloroform extract solution, conc. 0.1190 gm/ml) dimethylamin and the mixture was worked up as in 5.(i). 7.5 gm (84% yield) liquid which solidified on standing as crystals (m.p. 32 - 33°C) was thus obtained. This compound has camphor-like odour.

5.(v) ISOPROPYLAMIDOPHOSPHORODICHLORIDOTHIONATE:

Thiophosphoryl chloride (8.45 gm; 0.05 mole) in 50 ml chloroform was reacted with 5.9 gm (0.1 mole, 24.8 ml chloroform extract solution, conc. 0.2379 gm/ml) isopropylamine, and the mixture was worked up as in 5.(i). 8.0 gm (83% yield) white crystalline solid (m.p. 35 - 36°C) was thus obtained.

5. (vi) PYRROLIDINOPHOSPHORODICHLORIDOTHIONATE:

Thiophosphoryl chloride (8.45 gm, 0.05 mole) in 50 ml chloroform was reacted with 7.1 gm (0.1 mole) pyrrolidine in 20 ml chloroform, and the mixture was worked up as in 5.(i). 8.5 gm product was obtained

5. (vii) PIPERIDINOPHOSPHORODICHLORIDOTHIONATE:

Thiophosphoryl chloride (8.45 gm, 0.05 mole) in 50 ml chloroform was allowed to react with 8.5 gm (0.1 mole) piperidine in 20 ml chloroform. 9.0 gm product was obtained.

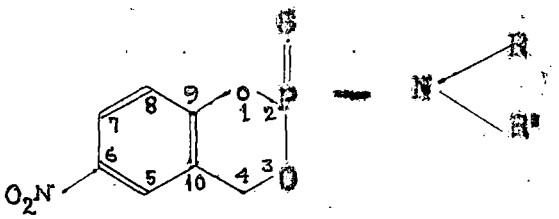
5.(viii) NONYLAMIDOPHOSPHORODICHLORIDOTHIONATE:

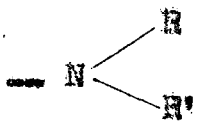
This compound was prepared by taking $PSCl_3$ (4.1 gm), triethylamine (2.46 gm, 0.0242 mole), and n - nonylamine (3.47 gm, 0.0242 mole) in 100 ml. chloroform; the nonylamine in 30 ml. Chloroform was added dropwise; after additional stirring for 3 hrs. the reaction mixture was washed twice with cold saturated sodium chloride solution. The chloroform phase was then dried with anhydrous sodium sulphate and filtered; evaporation in Vacuo gave 6.2 gm liquid nonylamidophosphorodichloridothionate.

5. (ix) HEPTADECYLAMIDOPHOSPHORODICHLORIDOTHIONATE:

Thiophosphorylchloride (1.32 gm, 0.0078 mole), triethylamine (0.79 gm, 0.0078 mole), and n - heptadecylamine (1.994 gm, 0.0078 mole) were used for the preparation of this compound; the heptadecylamine in 30 ml chloroform was added dropwise; 1.8 gm product was obtained.

6. PREPARATION OF 2 - ALKYLAMIDO - 6 - NITRO - 4H - 1,3,2 - BENZODIOXAPHOSPHORIN - 2 - SULPHIDE:



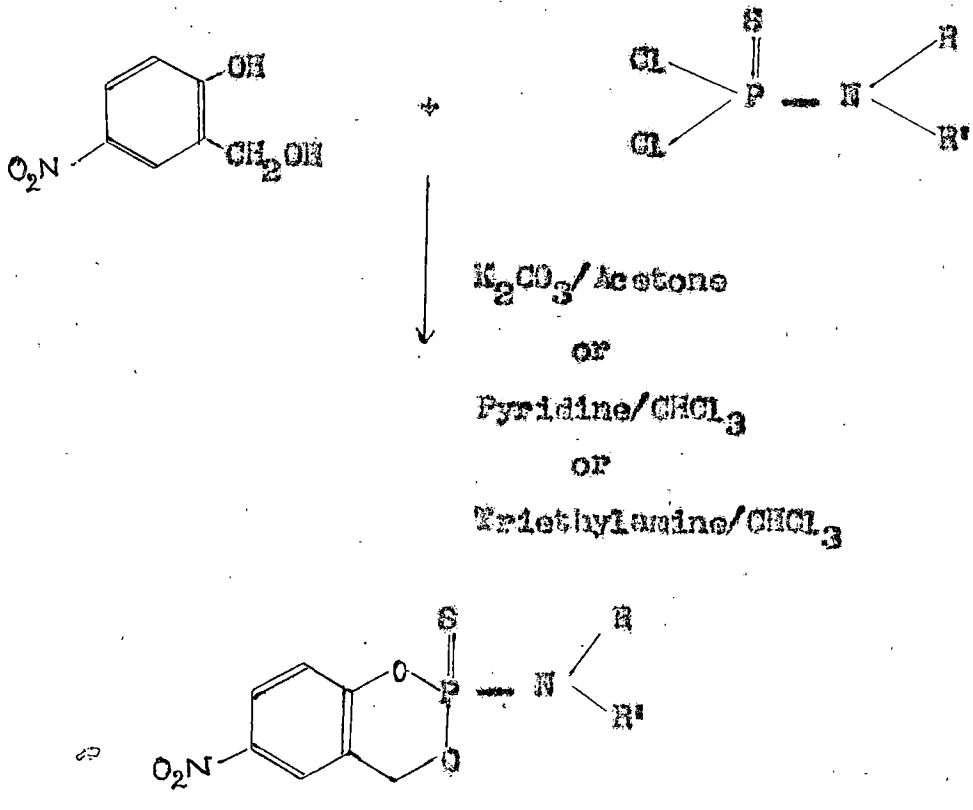


≡ Primary and Secondary amines.

6. (a) GENERAL PROCEDURE:

2-alkylamido - 6 - nitro - 4H - 1,3,2 - benzodioxaphosphorin - 2 - sulphides were prepared by adding a solution of 2 - hydroxy - 5 - nitrobenzyl alcohol (5 - nitro saligenin, 1 mole) in dry acetone to 1 mole of alkylamidophosphorodichloridothionate with cooling in an ice bath. The anhydrous potassium carbonate (2 mole) was then added by instalments, with constant stirring. In some cases, good result was found by using 2 moles of pyridine or 2 moles of triethylamine in chloroform solution instead of anhydrous potassium carbonate. The temperature of the reaction mixture was strictly maintained below 5°C during the addition of potassium carbonate/pyridine/triethylamine. The condensation was accomplished by stirring at the temperature 5 - 27°C for an additional time of 12 - 16 hrs. The solid particles were filtered out of the reaction mixture and the solvent was removed under reduced pressure at room temperature. In some cases the crude product was directly re-

crystallised from methanol to give the pure compound; while in others an additional chloroform extraction was necessary prior to the recrystallisation. In the latter case, the crude product was extracted with chloroform and washed with 1% dil HCl (ice - cooled) and with cold water, repeatedly (three times). This was then dried with anhydrous sodium sulphate and the chloroform was subsequently removed under reduced pressure. The pure compounds were then obtained by recrystallization from methanol.

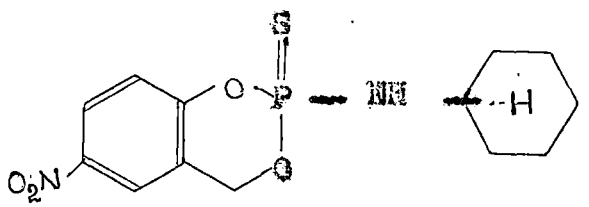


The different alkylamidophosphorothionates were prepared as follows:

6. (b) PREPARATION AND PROPERTIES OF SOME INDIVIDUAL ALKYLAMIDOPHOSPHOROTHIONATES:

Melting points are uncorrected, Micro analyses are carried out by Alfred Bernhardt, West Germany.

6. (b) (1) 2- CYCLOHEXYLAMIDO - 6 NITRO - 4H - 1,3,2 - BENZODIOXAPHOSPHORIN-2 - SULPHIDE (BD-10):



This compound (BD-10) was prepared by condensation of 5-nitro saligenin (1.69 gm; 0.01 mole) and cyclohexylamidophosphorodichloridothionate (2.32 gm, 0.01 mole) in presence of K_2CO_3 (2.76 gm; 0.02 mole) in 75 ml acetone as solvent; K_2CO_3 was added by instalments to the stirred solution at 0° to 5°C. After an additional stirring (2 - 3 hrs, at 0° to 5°C, and then 12 - 16 hrs. at room temperature), the solids were filtered off, and the solvent was removed. The crude product was washed with methanol saturated with n - heptane, and then the compound was recrystallised from hot methanol. 2.5 gm (76% yeild) compound was obtained. BD-10 is a white crystalline solid, m.p. 125°C, practically insoluble in water, highly soluble in methanol, ethanol and benzene.

Analysis

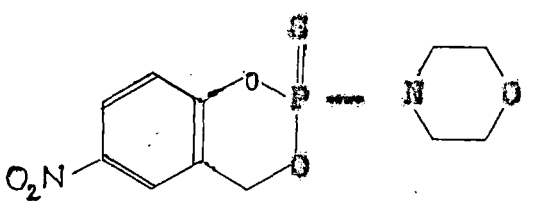
Found : C, 47.54% ; H, 5.19%; N, 8.52%;

Calculated for : $C_{13}H_{17}O_4N_2PS$: C, 47.56%; H, 5.18%, N, 8.53%.

Molecular Weight : 328

A better yield was obtained by using pyridine (chloroform as solvent) instead of K_2CO_3 as dehydrogen chloride agent.

6.(b)(ii). 2 - MORPHOLINO - 6 - NITRO - 4H - 1,3,2 - BENZODIOXAPHOSPHORIN - 2 - SULPHIDE (BD-11):



5 - Nitro - Saligenin (1.69 gm, 0.01 mole), morpholinophosphorodichloridethionate (2.2 gm, 0.01 mole), and K_2CO_3 (2.76 gm, 0.02 mole) in 50 ml acetone gave 3 gm (90% yield) crude product; this was then dissolved in chloroform and washed with cold dilute HCl and water; after drying with anhydrous sodium sulphate the solvent was removed under reduced pressure. The solid mass was then recrystallised from hot methanol. 2.0 gm (60% yield) BD-11 was thus obtained; BD-11 is a white crystalline solid, m.p. 149°C, highly soluble is acetone and chloroform, moderately soluble in methanol, ethanol and benzene.

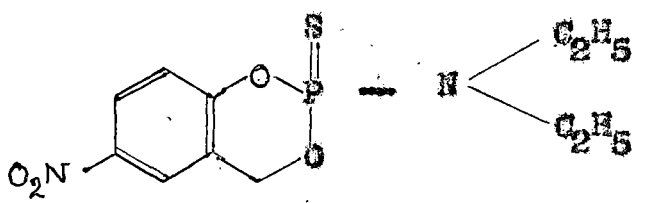
Analysis

Found : C, 41.75%; H, 4.10%; N, 9.24%.

Calculated for : $C_{11}H_{13}O_5N_2PS$: C, 41.77%; H, 4.11%; N, 9.27%.

Molecular Weight : 316.

6.(b)(iii). 2 - DIETHYLAMIDO - 6 - NITRO - 4H - 1,3,2 - BENZODIOXAPHOSPHORIN - 2 - SULPHIDE (BD-12):



5 - Nitro - Saligenin (1.69 gm, 0.01 mole), diethylamidophosphorodichloridothionate (2.06 gm, 0.01 mole), and K_2CO_3 (2.76 gm, 0.02 mole) in 50 ml acetone gave BD-12 (2.8 gm, 90% yield) as white crystals (after working up as in BD-10), m.p. 105°C.

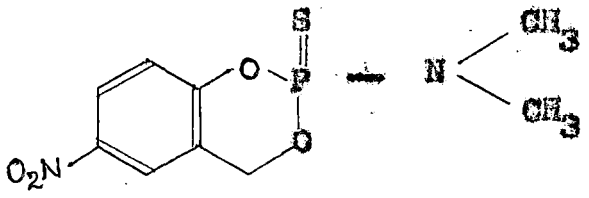
Analysis

Found : C, 43.68%; H, 4.94%; N, 9.25%.

Calculated for : $C_{11}H_{15}O_4N_2PS$; C, 43.70%; H, 4.96%; N, 9.27%

Molecular Weight : 302

6.(b)(iv). 2 - DIMETHYLAMIDO - 6 - NITRO - 4H - 1,3,2 - BENZODIOXAPHOSPHORIN - 2 - SULPHIDE (BD-13):



5 - Nitro - Saligenin (1.69 gm, 0.01 mole); dimethylamidophosphorodichloridothionate (1.78 gm, 0.01 mole), K_2CO_3 (2.76 gm, 0.02 mole) in 50 ml acetone gave BD-13 (2.0 gm, 75% yield) as white crystals (after working up as in BD-10) m.p. 128°C.

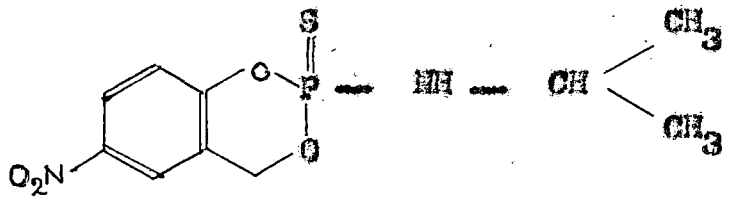
Analysis

Found : C, 39.40% ; H, 4.00%; N, 10.19%.

Calculated for : $C_9H_{11}O_4N_2PS$; C, 39.41%; H, 4.01%; N, 10.21%.

Molecular Weight : 274.

6.(b)(v). 2 - ISOPROPYLAMIDO - 6 - NITRO - 4H - 1,3,2 - BENZODIOXAPHOSPHORIN - 2 - SULPHIDE (BD-14):



5 - Nitro - Saligenin (1.69 gm, 0.01 mole) isopropylamidophosphorodichloridothionate (1.92 gm, 0.01 mole), K_2CO_3 (2.76 gm, 0.02 mole in 50 ml acetone gave BD-14 (2.0 gm, 70% yield) as white crystals, m.p.

98°C.

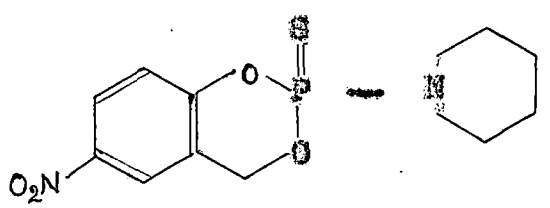
Analysis

Found : C, 41.64%; H, 4.50%; N, 9.70%;

Calculated for : $C_{10}H_{13}O_4N_2PS$: C, 41.66%; H, 4.51%; N, 9.72%;

Molecular Weight : 288

6.(b)(vi). 2 - PYRROLIDINO - 6 - NITRO - 4H - 1,3,2 - BENZODIOXAPHOSPHORIN - 2 - SULPHIDE (BD-15):



5 - Nitro - Saligenin (2.70 gm, 0.016 mole), pyrrolidinophosphorodichloridothionate (3.45 gm, 0.016 mole), K_2CO_3 (4.5 gm, 0.032 mole) in 50 ml acetone gave BD-15 (4 gm, 83% yield) as white crystals, m.p. 134°C.

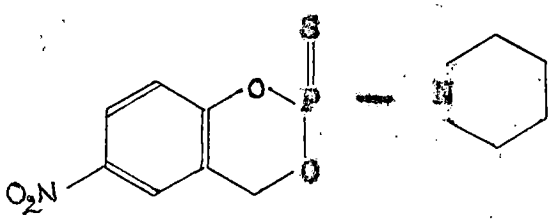
Analysis

Found : C, 43.82%; H, 4.27%; N, 9.28%.

Calculated for : $C_{11}H_{13}O_4N_2PS$: C, 43.91%; H, 4.32; N, 9.32%.

Molecular Weight : 300.6

6.(b) (vii). 2 - PIPERIDINO - 6 - NITRO - 4H - 1,3,2 - BENZODIOXAPHOSPHORIN - 2 - SULPHIDE (BD -16):



5 - Nitro - Saligenin (3.4 gm, 0.02 mole), piperidinophosphorodichloridothionate (4.36 gm, 0.02 mole), K_2CO_3 (5.5 gm 0.04 mole) in 50 ml acetone gave BD-16 (5.5 gm, 87% yield) as white crystals, m.p. 130°C.

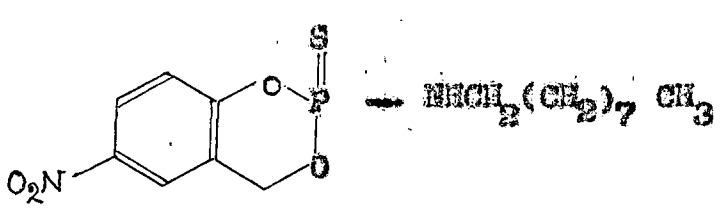
Analysis

Found : C, 45.72%; H, 4.71%; N, 8.82%.

Calculated for ; $C_{12}H_{15}O_4PS$; C, 44.71%; H, 4.77%; N, 8.90%.

Molecular Weight : 314.6

6. (b) (viii). 2 - NONYLAMIDO - 6 - NITRO - 4H - 1,3,2 - BENZODIOXAPHOSPHORIN - 2 - SULPHIDE (BD-17):



5 - Nitro - Saligenin (1.7 gm, 0.0108 mole) nonylamidophosphorodichloridothionate (2.98 gm, 0.0108 mole), K_2CO_3(2.9 gm, 0.0216

mole) in 50 ml acetone gave BD-17 (3.5 gm, 87% yield) as crystals, m.p. 64°C.

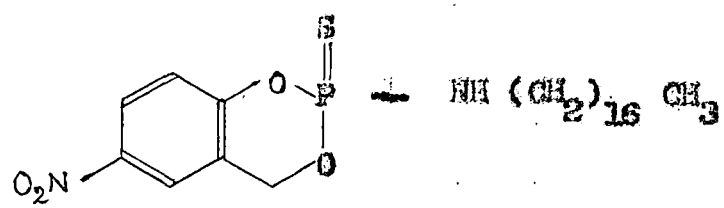
Analysis

Found : C, 51.58%; H, 6.69%; N, 7.60%.

Calculated for : $C_{16}H_{25}O_4N_2PS$: C, 51.61%; H, 6.72%; N, 7.53%.

Molecular Weight : 372.

6.(b)(ix). 2 - HEPTADECYLAMIDO - 6 - NITRO - 4H - 1,3,2- BENZODIOXAPHOSPHORIN - 2 - SULPHIDE (BD-18):



5 - Nitro - Saligenin (0.78 gm, 0.0046 mole), heptadecylamidophosphorodichloridothionate (1.8 gm, 0.0046 mole), triethylamine (0.93 gm) in 50 ml chloroform gave BD-18 (1.82 gm) as crystals, m.p. 70°C.

Analysis

Found : C, 59.46 ; H, 8.41 ; N, 5.76.

Calculated for : $C_{24}H_{41}O_4N_2PS$: C, 59.50 ; H, 8.47; N, 5.78

Molecular Weight : 484.

7. FUNGICIDAL ACTIVITY:

7. (a) TEST ORGANISM:

Pyricularia Oryzae Cav. - causal fungus of blast disease of rice.

7. (b) CULTURE MEDIUM :

Solid medium : Malt extract agar.

20 gm malt (Difco) extract was boiled in water till dissolved. 20 gm agar agar (Kobe Japan) was added and boiled until agar was well dissolved. 0.05 gm chloramphenol 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 minutes.

7. (c) GROWTH INHIBITION METHOD:

Growth inhibition studies were made by using poison food technique[4]. Acetone solution of suitable quantity of the compounds in sterile water containing 0.01 percent Triton - X 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 mm 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 $\pm$ 1°C in dark. Linear growth of the fungal disc were measured after 24,48,72 and 96 hours interval

Percent inhibition over control was calculated following the equation given by Vincent[5].

8. INSECTICIDAL ACTIVITY ON GRASSHOPPERS:

Insecticidal tests were also performed on the Sporadic Grasshoppers, Oxya nitidula by topical application of acetone solution of the compounds according to Eto et al[6]. The grasshoppers weighing about 0.15 to 0.25 gm, were collected in the month of October - November, 1981 from one particular location of the North Bengal University Campus. In the field they were never exposed to any Organophosphorus Insecticides. For preliminary experiment, acetone solution of the compound was topically applied to ten insects in each pot, and after 24 hours the mortality was determined.

To determine the more precise LC_{100} value of each compound, one insect of known weight in each pot was treated, by topical application, with acetone solution of test chemicals on mouthband thoracic region (Ventral side) of the insect. Each test was triplicated. For all the alkylamidophosphorothionates the concentration was increased by 1 μg/insect, but for the salithion (standard) the concentration was increased by 0.1 μg/insect.

9. ACUTE ORAL TOXICITY TESTS ON RATS:

Oral toxicity testing was conducted on 6 - 12 months old male white albino rats, weighing 140 - 200 gm, 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[7] feeding time. The mortality within

48 hours 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 animal during dieting was roughly accounted for in determining the dosage.

10. PHYTOTOXICITY TESTS:

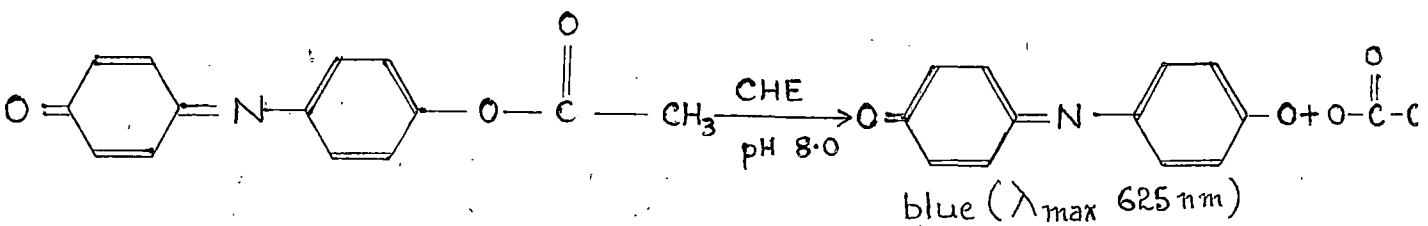
Phytotoxicity testing was conducted according to Eto et al.(8) Acetone solution of the compounds mixed with fixed amount of water containing 0.01%. Triton - X was prepared. 5 ml of this aquous suspension containing 500, 250 or 100 ppm of the compounds was poured into a petridish bottom covered with absorbent cotton. Ten seeds of wheat (Triticum sp UP 262 variety supplied by National Seed Corporation of India) were placed on the cotton and kept at room temperature (25 - 27°C) for five days. Occasionally 2 - 4 ml water was added in each petridish so that the seeds remained in moist condition. Each test was triplicated. Number of germination was counted after 5 days.

11. ANTICHOLINESTERASE ACTIVITY:

The organophosphorus compounds have a common pharmacological property which is the ability to inhibit the activity of a group of enzymes, especially acetyl cholinesterase (AChE), involved in the hydrolysis of esters of choline. Since these enzymes present widely in insects and mammals, the organophosphorus compounds, used as insecticides also exhibit high mammalian toxicity.

11. (a) ANTICHOLINESTERASE ACTIVITY IN HOUSEFLY - HEAD HOMOGENATE:

The acetyl cholinesterase inhibition of housefly head brie (HFAChE) has been measured by colorimetric method of Kramer and Gamson[9,10], using indophenyl acetate as an internal substrate-indicator in 0.05 M phosphate bubber of pH 8.0. The enzymatic reaction for indophenyl acetate is as follows:



The reaction mixture contained 5 ml buffer containing housefly head brie (1 ml of this solution contained 1 fly head) and 0.15 ml indophenyl acetate (total volume = 5.15 ml, conc. of indophenyl acetate $\sim 10^{-5}$ M). The readings for control and sample were taken at 625 nm after exactly 30 minutes incubation.

11. (a) (i) REAGENTS:

1. Buffer solution : (0.05 M potassium dihydrogen phosphate): Clark and Lubs buffer of pH 8.0:

6.8 gm KH_2PO_4 dissolved in 500 ml of water was mixed with 475 ml of 0.1 N NaOH solution and diluted to litre after the pH was adjusted to 8.0.

2. Indophenyl acetate : Working solution:

0.008 gm of indophenyl acetate when dissolved in 10 ml of

absolute ethyl alcohol gave a 3.3×10^{-3} M solution so that the final concentration of the indophenyl acetate in the reaction vessel was always 9.6×10^{-5} M.

3. Glycerol solution:

10 ml of Glycerol was diluted to 100 ml with absolute alcohol.

4. Saline solution : 0.9%

9 gm of NaCl was dissolved in 1 litre distilled water

5. Salt solution:

2.03 gm of Manganous chloride and 2.15 gm of NaCl were dissolved in 250 ml of water.

6. Preparation of working solution of acetylcholinesterase from housefly-heads:

About 500 houseflies (Musca domestica) closed in a glass vessel was stored in a deep freeze for 1 - 2 hours. They were then transferred in a container with finely broken dry ice, removed individually from the container, decapitated with a shaving blade and forceps. 400 heads were combined with 2 ml of salt solution and 2 gms of washed sand in a prechilled with size No. 1 morter. The heads were slowly ground, then transferred to 50 ml plastic centrifuge tube with one 3 ml aliquots of cold saline solution and two 5 ml aliquots of buffer solution. The head fragments were removed by centrifugation for

10 minutes at 10.000 r.p.m. in super speed centrifuge at 4°C. The supernatant liquid was decanted into a graduated cylinder and the fragmented heads were mixed with 10 ml of buffer solution and centrifuged again at 10.000 r.p.m. This extraction procedure was repeated twice. The supernatant solutions were combined and the volume was adjusted to 80 ml with the buffer solution so that each ml was equivalent to 5 fly-heads. This solution was stocked frozen in deep freeze. One ml of this solution was diluted to 5 ml with buffer solution so that each ml of the diluted solution contained single fly-head and used for each set of the experiment.

11.(a)(ii) METHOD:

A series of 15 ml pyrex beakers (numbered 1,2,3..... etc.) containing different amounts of inhibitor (Viz, BD-10, BD-11/etc.) in acetone along with one marked 'control' without inhibitors were arranged. 0.6 ml of glycerol solution was poured in each beaker including the 'control'. The acetone (in beaker 1,2,3 etc.) was removed by blowing cold air.

To the 'control' 5 ml of working enzyme - buffer solution was added and simultaneously the stop-watch was started. At the interval of exactly 2 minutes, 5 ml of the enzyme-buffer solution was added to each of the remaining beakers.

After exactly 30 minutes, 0.15 ml of the indophenyl acetate 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 minutes and then kept to be incubated at 30°C. After incubation for

exactly 30 minutes the absorbances of 'control' and remaining solution were successively noted in the spectrophotometer (Carl - Zeiss Specol, ZV) at 625 nm with reference to enzyme-buffer (reagent blank) solution.

Calculation

$$\% \text{ inhibition} = \frac{\text{Absorbance (Control)} - \text{Absorbance (Sample)}}{\text{Absorbance (Control)}} \times 100$$

12. 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 [9,10], using indophenyl acetate as an internal substrate indicator in 0.05 M 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 indophenyl acetate in the reaction mixture $\simeq 10^{-5}$ M). The readings of 'control' and 'sample' were taken at 625 nm, after exactly 30 minute incubation.

12. (a) MATERIALS AND METHODS:

(1) Materials:

(a) Goat whole blood: 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^{o}C.

The other reagents e.g. (b) indophenylacetate (c) phosphate buffer solution and (d) glycerol solution were prepared just as the same as mentioned in 11 (a).

(11) Methods:

The anticholinesterase activity in goat whole blood was determined by the same method as describe in 11 (a). The only exception was that the contents of the beaker was filtered through a 4.25 cm whatman No-1 filter paper after exactly 29 minutes incubation and the absorbance of the filtrate of the enzyme buffer solution and % inhibition was calculated in the same way as describe in 11 (a).

13. CHEMICAL HYDROLYSIS:

The chemical hydrolysis studies were performed in 0.0095 M NaOH solution in 60 percent ethanol of pH 11.85 at 20°C. One ml of ethanol solution of the compounds was added with 9 ml of 0.0095 M NaOH solution (total volume 10 ml). The rate of hydrolysis was monitored by following the formation of nitro-saligenin anion at 410 nm in a Carl Zeiss Speckol ZV spectrophotometer. UV spectra of the alkylamidophosphorothionates and hydrolytic product were examined prior to Kinetic studies to show that overlap in relevant absorption peaks were not present. The concentration co-efficient value of 5 - nitro saligenin in the same 0.0095 M NaOH solution at the said wave lengths. The pseudo first order rate constants (K_{hyd}) were determined by the least square regression analysis.

14. STUDIES ON FUNGICIDAL ACTIVITY USING SPORE GERMINATION METHOD:

14.(a) TEST ORGANISM:

1. Helminthosporium oryzae, Breda de Haan - causal organism of brown leaf spot disease of rice.

2. Verticillium albo-atrum. Reinke and Berthhold — causal organism of stock rot disease of banana.

3. Aspergillus niger. Van Tiegh - causal organism of rot of stored grains.

4. Penicillium gensemi. -causal organism of rot of stored grains.

14.(b) CULTURE MEDIUM:

Liquid medium : Malt solution.

40 gm. malt (Difco) extract was boiled in water till dissolved. 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. The medium was then sterilized at 15 p.s.i. for 20 minutes.

14.(c) SPORE GERMINATION METHOD:

Spore germination studies were made by using slide germination technique (11). Acetone solution of the compound was diluted

by sterile water in order to get the required amount of the compound in water suspension. Spore suspension of the fungus was prepared by flooding the surface of 12 to 15 days old culture with 5 ml sterile water; for A. niger and P. gensenl 0.01% agar solution was used in place of sterile water. Density of spore suspension in the final solution was determined by using a haemocytometer, and the final concentration was maintained at 20,000 spore/ml. Slides having spores in compound suspension were incubated at 30° ± 1° C and observation of the conidial germination were recorded after 24 and 48 hours. Three replication of each fungicidal concentration were maintained. A total of 300 spores from three replications were counted and percent inhibition of the spore germination was calculated against appropriate control.

15. FURTHER STUDIES ON FUNGICIDAL ACTIVITY ON OTHER FUNGI USING GROWTH INHIBITION METHOD:

Test organism:

1. Helminthosporium oryzae Breda de Haan;

2. Alternaria solani (Ell and Mart) Jones and Grout - causal organism of early blight disease of potato.

3. Verticillium albo-atrum. Reinke and Berthhold;

The method used was stated previously [7 (c) - page - 109-110].

16. STUDIES (in vivo) OF FUNGICIDAL ACTIVITY OF SOME COMPOUNDS AGAINST H. oryzae ON RICE PLANT:

An isolate of H. oryzae was obtained from the Indian Agricultural Research Institute (New Delhi) and the culture was maintained on potato - dextrose-agar medium. Seeds of Dharial, a cultiver of rice susceptible to brown leaf spot disease, were used in the experiments. Seeds of this cultiver were grown at the Rice Research Station, Chinsurah (West Bengal). Naturally infected seeds of the same cultiver were also collected from the station and hand screened to give only spotted, infected seeds. Seventy to 75% of the spotted seeds yielded H. oryzae when grown in PDA.

16.(a) Determination of protectant activity on detached rice leaves:

Rice plants were grown in earthenware pots containing compost-enriched soil. When the plants were 40 days old, leaves of approximately the same age were washed, dried, removed from the plants and placed over moistened blotting paper taken in large petridishes. The required amount of compound in water suspension was sprayed on the leaves and allowed to dry for 15 to 20 minutes. Drops of conidial suspension of H. oryzae (8×10^4 spore/ml) prepared from 15 days old slant cultures were placed over the surface of the treated and untreated leaves. The petridishes were covered with polythene sheets and incubated at 21 - 23°C. After 48 hours production of lesion on leaves was recorded; percent inhibition of infection was calculated according to Chattopadhya and Bose (12).

16.(b) Protectant activity on rice seedlings:

Forty - day - old plants were sprayed almost to run off with water suspension of the compounds. Plants sprayed with water served as controls. After 12 hours, plants were inoculated by spraying with a conidial suspension of H. oryzae (8×10^4 conidia/ml). The inoculated plants were kept for 48 hours in a room in which high relative humidity and a temperature of 23 - 25° C were maintained. After incubation, the plants were placed in a greenhouse. The disease index was calculated after 6 days by using a modification of the method of Sinha and Trivedi[13]. The number and size of the spots appearing on leaves were recorded 6 days after incubation. The spots were graded into three size groups; small (upto 2.5 mm), medium (2.5 mm to 5 mm) and large (above 5 mm), with respective values of 0.25, 0.5 and 1.0 assigned to them. The number of spots in each size group was multiplied by the value assigned to it and the sum total of such values for all of the leaves gave the disease index for a plant[12].

17. FURTHER STUDIES ON PHYTOTOXICITY OF SOME OF THE COMPOUNDS:

In this experiment above mentioned Dharial variety rice seeds have been used.

17. (a). Effect of treatment on rice seeds:

Well dried, cleaned seeds were soaked in acetone-water suspension of different compounds in two lots - one for two hours, and another for four hours respectively. The seeds were then dried in

open air. The seed germination were carried out on the next day in sterile petridishes containing moist filter paper. Hundred seeds were tested in each treatment and the germination percentage were observed and recorded. The germinated seeds were allowed to grow in petridishes for 7 days. The root and shoot length were recorded on 7th day. A parallel set of control was maintained always.

17. (b) Effect of treatment on rice plants:

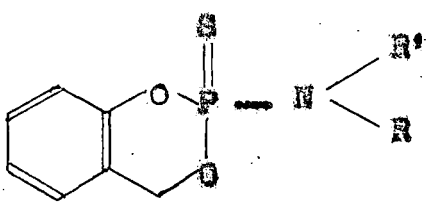
Thirty-day-old rice plants were sprayed almost to run off with water suspension of the compounds; the spraying was continued for consequtive 6 days (once daily). Each pot containing 10 plants were treated seperately. Each experiment was done with five replications. A suitable control was maintained all along. The shoot length was measured before spraying (on 31 day) and after final completion of spraying (37th day).

B. RESULTS AND DISCUSSION

B. RESULTS AND DISCUSSION:

1. SYNTHESIS

Only four saligenin cyclic phosphoramidothionates have so far been reported[14]. The physical properties and percent yield of these compounds are given below.

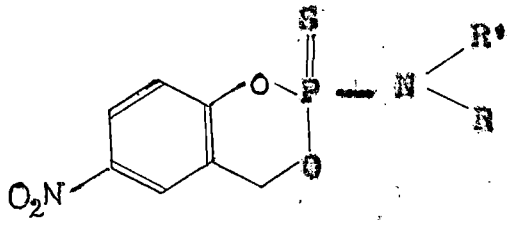


Amido Group	Code No.	Yield percent	b.p. °C/mm Hg.
Methylamido	K - 35	20	120 - 123/0.2
Ethylamido	K - 37	--	Undistillable liquid
Dimethylamido	K - 36	27	118 - 122/0.2
Diethylamido	K - 38	13	110/0.2

All of them are liquids, and percent yield are low (13 - 27%)[14].

We, however, succeeded to prepare the nitro-saligenin cyclic phosphoramidothionates (BD-10 to BD-13) in solid crystalline form with high yields (Table - 1).

Table - I



Code No.	Amido Group	Yield (%)	m.p. (°C)
BD - 10	Cyclohexylamido	70 - 90	126
BD - 11	Morpholino	60 - 80	149
BD - 12	Diethylamido	80 - 90	105
BD - 13	Dimethylamido	75 - 80	128
BD - 14	Isopropylamido	70 - 80	98
BD - 15	Pyrrolidino	80 - 90	134
BD - 16	Piperidino	80 - 90	130
BD - 17	Nonylamido	80 - 90	64
BD - 18	Heptadecylamido	80 - 90	70

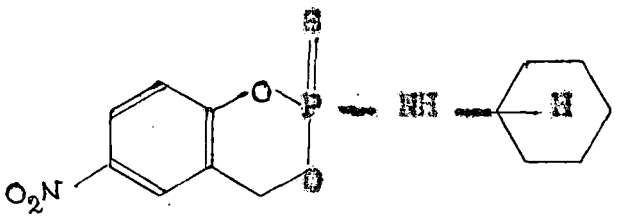
Attempts had also been made several times to prepare the 2-methylamido-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-sulphide, and its ethylamido analog; unfortunately, in each case a viscous yellowish liquid was obtained. TLC of these liquids gave several spots indicating the presence of a number of compounds; the pure amidophosphorothionates could not be isolated from these liquid mixtures.

2. STRUCTURE DETERMINATION BY SPECTROSCOPIC METHOD:

The structure of the compounds have been determined by chemical analysis and IR, mass and NMR spectra. The analytical data have been presented in Section - A. The spectral data of only four compounds are given below:

2.(a) SPECTRAL DATA

(1) 2-Cyclohexylamido-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-sulphide (BD-10):



IR (Fig. 1). (cm^{-1}):

650 (m), 740 (m), 800 (s), 880 - 920 (s), 1020 (s), 1240 (vs), 1340 (s), 1420 (m), 1480 (m), 1515 (s), 1584 (m), 1620 (w) and 3300 (m).

Mass (Fig.2):

m/e	328 (M^+),	296,	295 (base peak),	279,	249
% RI	34.5,	43.5,	100	, 46.0,	33.0

m/e	230 ,	198	, 152
%RI	36.0,	54.5	, 60.0

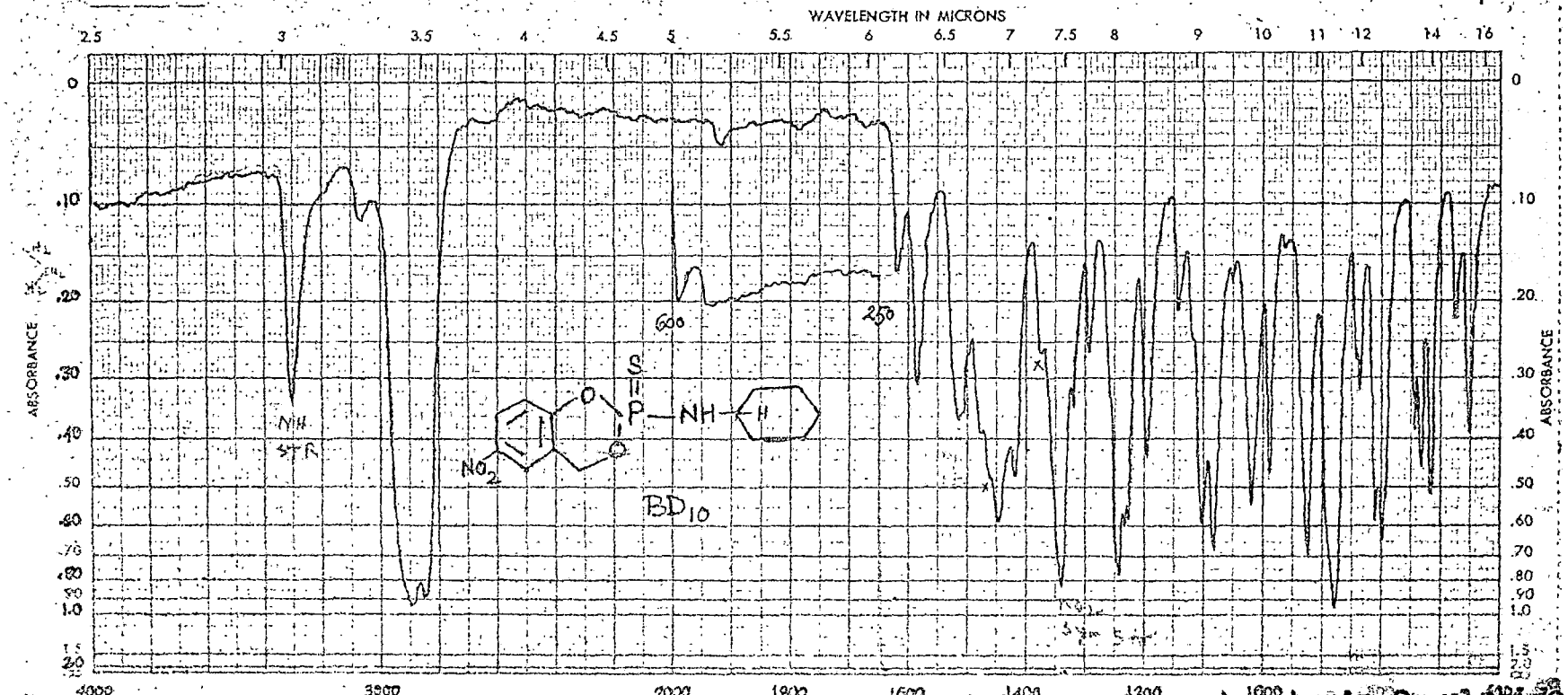


Fig.-1 IR-spectra of 2-Cyclohexylamido-6-nitro-4H-1,3,2 benzodioxaphosphorin-2-sulphide.

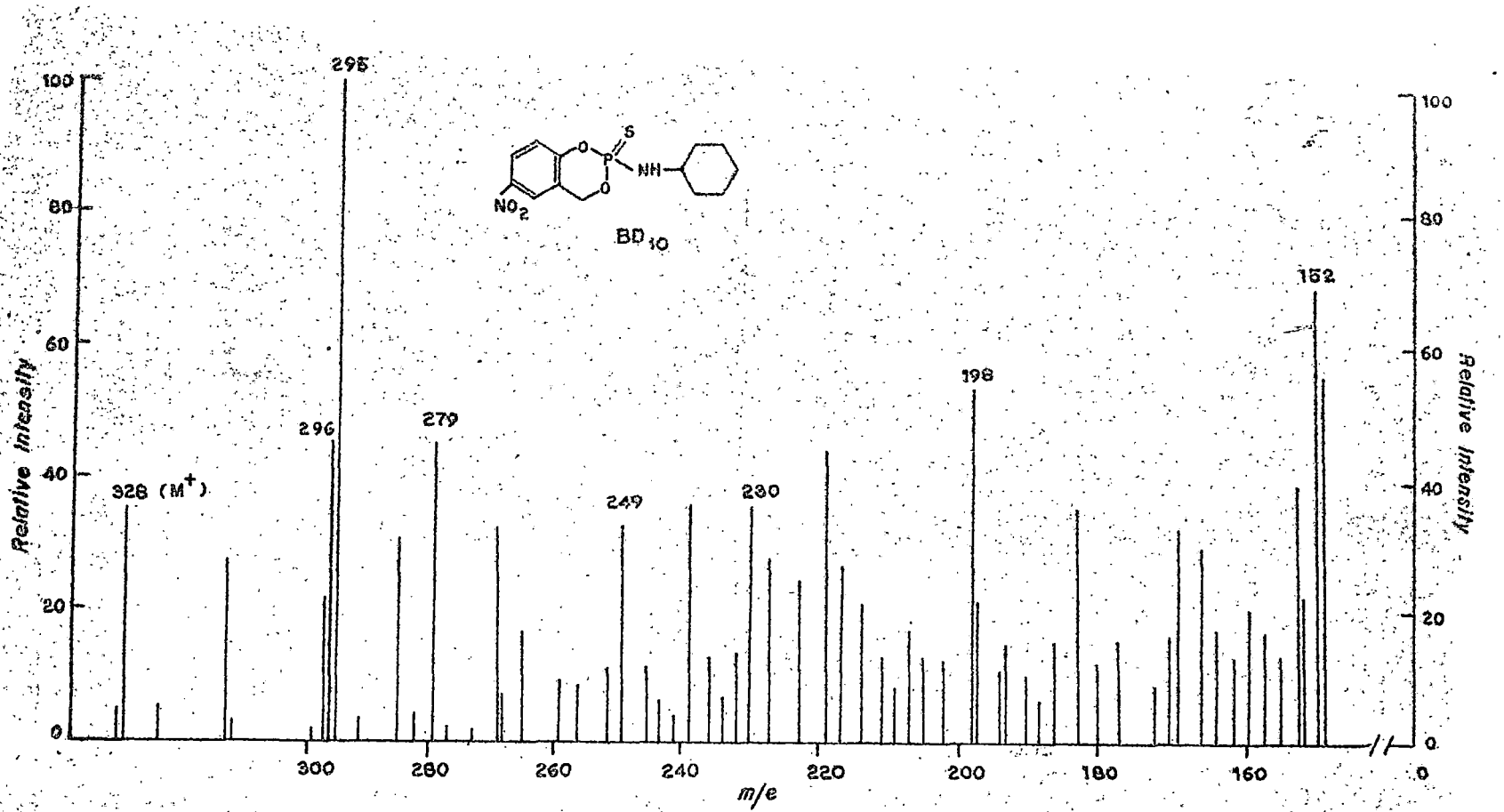


Fig.-2 Mass spectra of -2- Cyclohexylamido - 6- nitro 4H- 1,3,2 benzodioxaphosphorin-2- sulphide.

^1H NMR δ ($CDCl_3$) ppm (Fig.3):

1.25 (2H, multiplet, $-CH_2-$group at 4 position of the cyclohexylamin);

1.6 (4H, multiplet, two-CH_2-groups, at 3,3' position of the cyclohexylamin);

1.9 (4H, multiplet two-CH_2-groups at 2,2' position of the cyclohexylamin);

3.2 (1H, multiplet $-CH<$ group);

3.6 (1H, multiplet -P-NH- group);

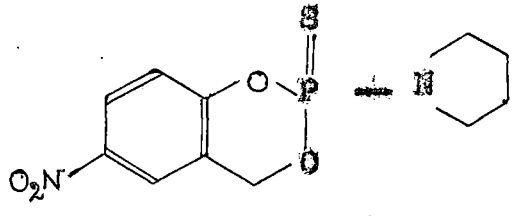
5.45 (2H, 8-liner multiplet $-CH_2-$ group in dioxaphosphorin ring);

7.1 (1H, doublet, J = 8.6 H_2, one aromatic hydrogen meta to nitro group);

8.06 (1H, doublet, one aromatic hydrogen ortho to both nitro group and $-CH_2-$ group of dioxaphosphorin ring);

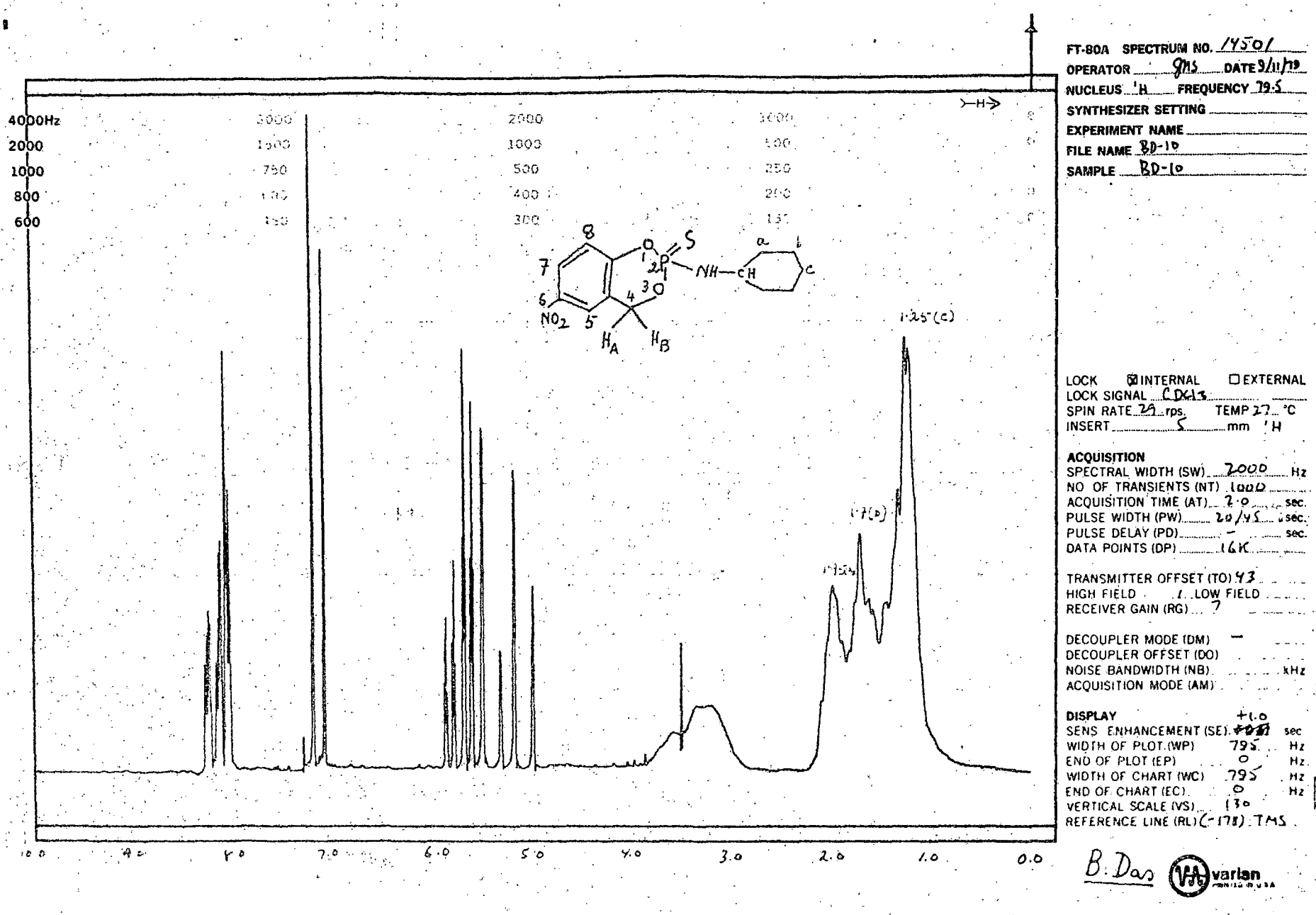
8.2 (1H, multiplet, remaining one aromatic hydrogen).

(ii) 2 - Pyrrolidino-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-sulphide (BD-15):



IR (Fig.4). (cm^{-1}):

655 (s), 735 (m), 810 (s), 875 (s), 1030 (s), 1250 (s), 1340 (vs) and 1520 (vs).



FT-80A SPECTRUM NO. 1450/
OPERATOR BMS DATE 9/11/79
NUCLEUS ^1H FREQUENCY 79.5
SYNTHESIZER SETTING
EXPERIMENT NAME
FILE NAME BD-10
SAMPLE BD-10

LOCK ☒ INTERNAL ☐ EXTERNAL
LOCK SIGNAL CDCl3
SPIN RATE 20 rps. TEMP 27 °C
INSERT 5 mm ^1H

ACQUISITION
SPECTRAL WIDTH (SW) 2000 Hz
NO OF TRANSIENTS (NT) 1000
ACQUISITION TIME (AT) 2·0 sec.
PULSE WIDTH (PW) 20/45 µsec.
PULSE DELAY (PD) — sec.
DATA POINTS (DP) 16K

TRANSMITTER OFFSET (TO) 43
HIGH FIELD / LOW FIELD
RECEIVER GAIN (RG) 7

DECOUPLER MODE (DM) —
DECOUPLER OFFSET (DO)
NOISE BANDWIDTH (NB) kHz
ACQUISITION MODE (AM)

DISPLAY +1.0
SENS ENHANCEMENT (SE) sec
WIDTH OF PLOT (WP) 795 Hz
END OF PLOT (EP) 0 Hz
WIDTH OF CHART (WC) 795 Hz
END OF CHART (EC) 0 Hz
VERTICAL SCALE (VS) 130
REFERENCE LINE (RL) (-178) TMS

B. Das varian

Fig. 3 ^1H NMR Spectra of 2-Cyclohexylamido-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-sulphide.

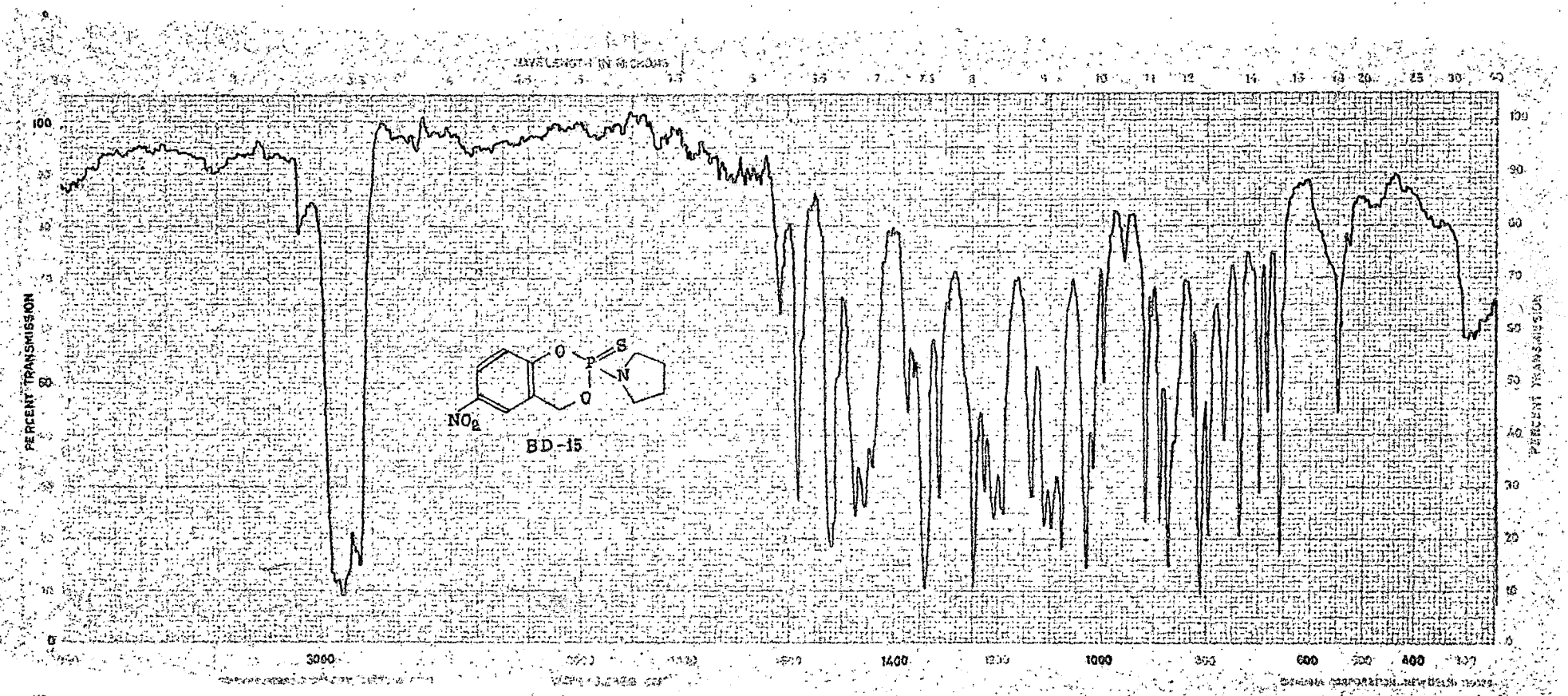


Fig. 4. IR Spectra of 2-Pyrrolidino-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-sulphido.

Mass (Fig. 5):

m/e	300 (M^+) ,	267 ,	198 ,	148 ,	116 ,	70
%RI	21.4 ,	100 ,	26.7 ,	11.4 ,	80.0 ,	35.7

^{1}H NMR δ ($CDCl_3$) ppm (Fig.-6):

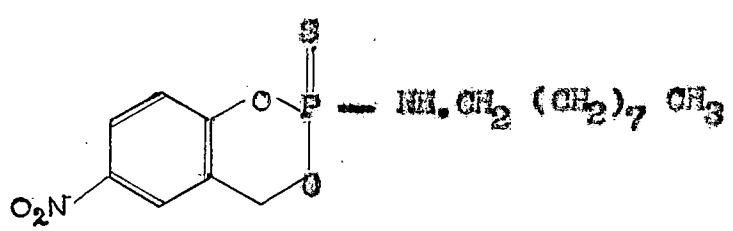
1.62 - 1.95 (4H, multiplet, two $-CH_2-$groups at 2, 2' positions of the pyrrolidine ring);

3.36 (4H, multiplet, two $-CH_2-$ groups adjacent to nitrogen);

5.26 and 5.66 (2H, $-CH_2-$ group in dixaphosphorin ring);

7.06, 8.0 and 8.14 (due to aromatic hydrogens).

(111) 2- Nonylamido-6-nitro-4H.1,3,2-benzodioxaphosphorin-2-sulphide (BB.17):



IR.(Fig-7). (cm^{-1}):

660 (s), 740 (m), 810 (s), 880 to 895 (s), 1030 (s), 1250 (s), 1340 (s), 1520 (s), 1585 (m), 1620 (v) and 3310 (s)

Mass (Fig-8)

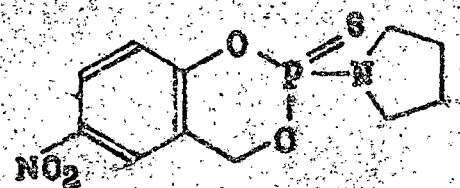
m/e,	372 (M^+) ,	339 ,	259 ,	230 ,	198 ,	152
%RI,	21.4 ,	100 ,	15.7 ,	7.1 ,	20 ,	10

^{1}H NMR δ ($CDCl_3$) ppm (Fig-8A): 0.9, 1.2. 3.0, 3.4, 3.6, 5.5, 7.05, 8.0, 8.2.

SPEKTRUM 23 VERDAMPFUNGSTEMPERATUR 120 GRAD
MOLEKUELPEAK: 300
MASSEN CHARAKTERISTISCHER IONEN:
267=300-SH

ANALYSE: 62907

STH HA 015 00
ST STEENKEN
MESSG:
AUSW :25-MAR-81
AUSWER: SCH



BD-15

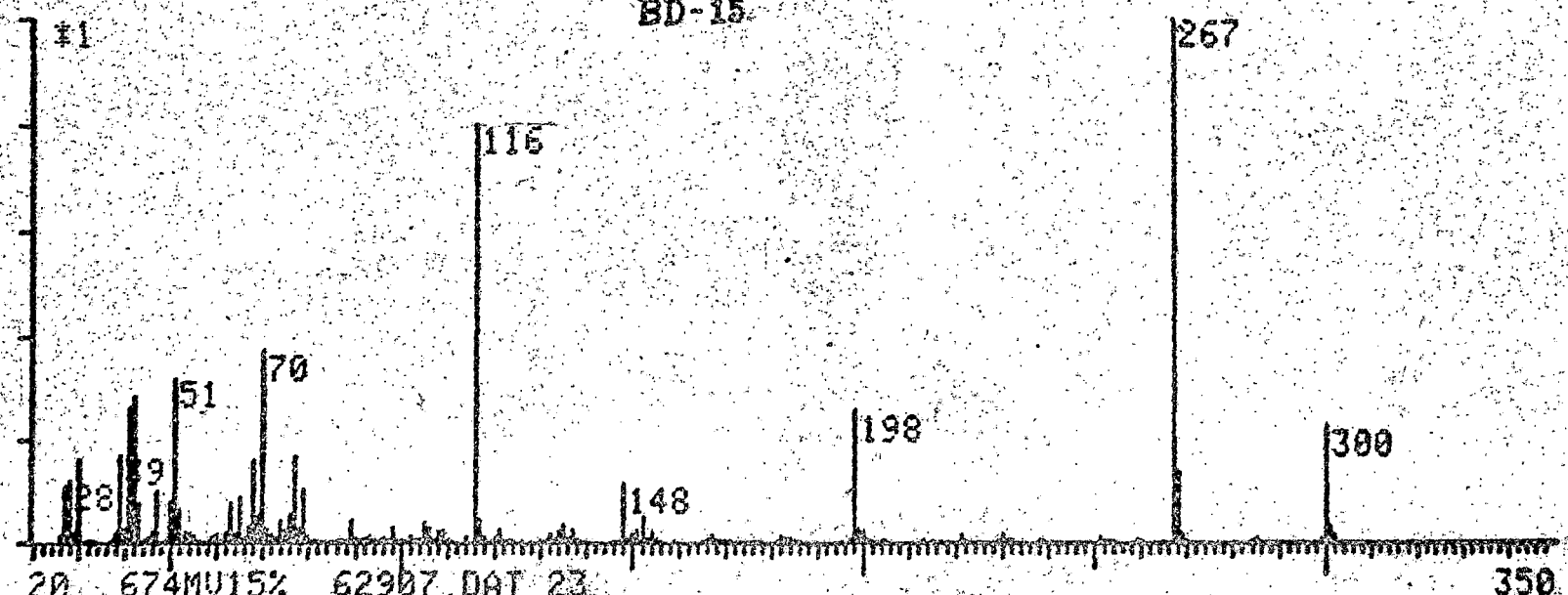
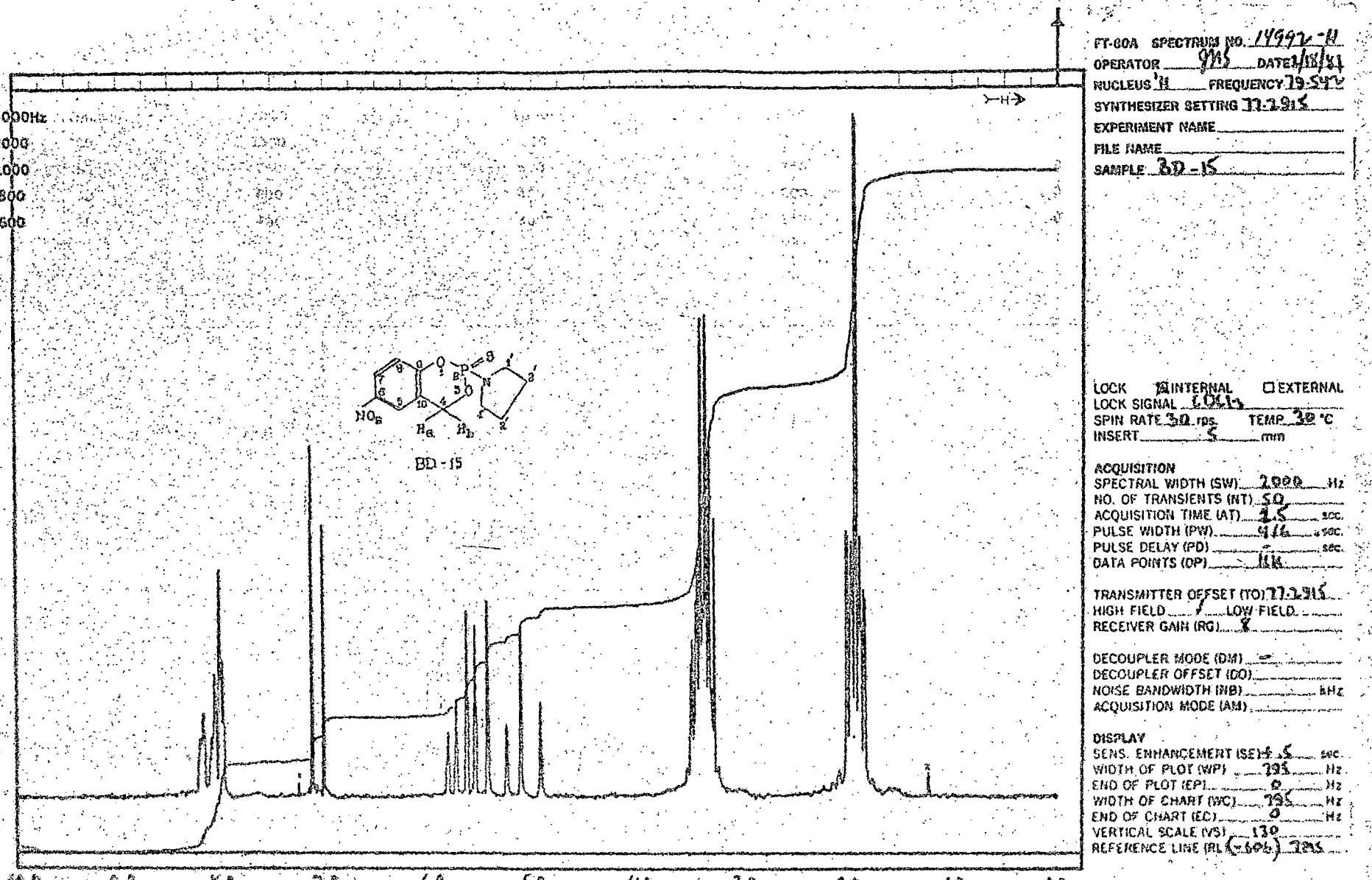


Fig. 5. Mass Spectra of 2-Pyrrolidino-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-sulphide.



FT-60A SPECTRUM NO. 14992-11
OPERATOR JMS DATE 1/18/81
NUCLEUS ^1H FREQUENCY 19.547
SYNTHESIZER SETTING 11.2915
EXPERIMENT NAME
FILE NAME
SAMPLE BD-15

LOCK ☒INTERNAL ☐EXTERNAL
LOCK SIGNAL LOCK
SPIN RATE 30 rps TEMP 30 °C
INSERT 5 mm

ACQUISITION
SPECTRAL WIDTH (SW) 2000 Hz
NO. OF TRANSIENTS (NT) 50
ACQUISITION TIME (AT) 1.5 sec.
PULSE WIDTH (PW) 4½ µsec.
PULSE DELAY (PD) 1 sec.
DATA POINTS (DP) 16K

TRANSMITTER OFFSET (TO) 11.2915
HIGH FIELD / LOW FIELD
RECEIVER GAIN (RG) 8

DECOUPLER MODE (DM)
DECOUPLER OFFSET (DO)
NOISE BANDWIDTH (NB) Hz
ACQUISITION MODE (AM)

DISPLAY
SENS. ENHANCEMENT (SE) 1.5 sec.
WIDTH OF PLOT (WP) 795 Hz
END OF PLOT (EP) 0 Hz
WIDTH OF CHART (WC) 795 Hz
END OF CHART (EC) 0 Hz
VERTICAL SCALE (VS) 130
REFERENCE LINE (RL) (-606) 795

varian

Fig. 6 ^1H NMR Spectra of 2-Pyrrolidino-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-sulphides.

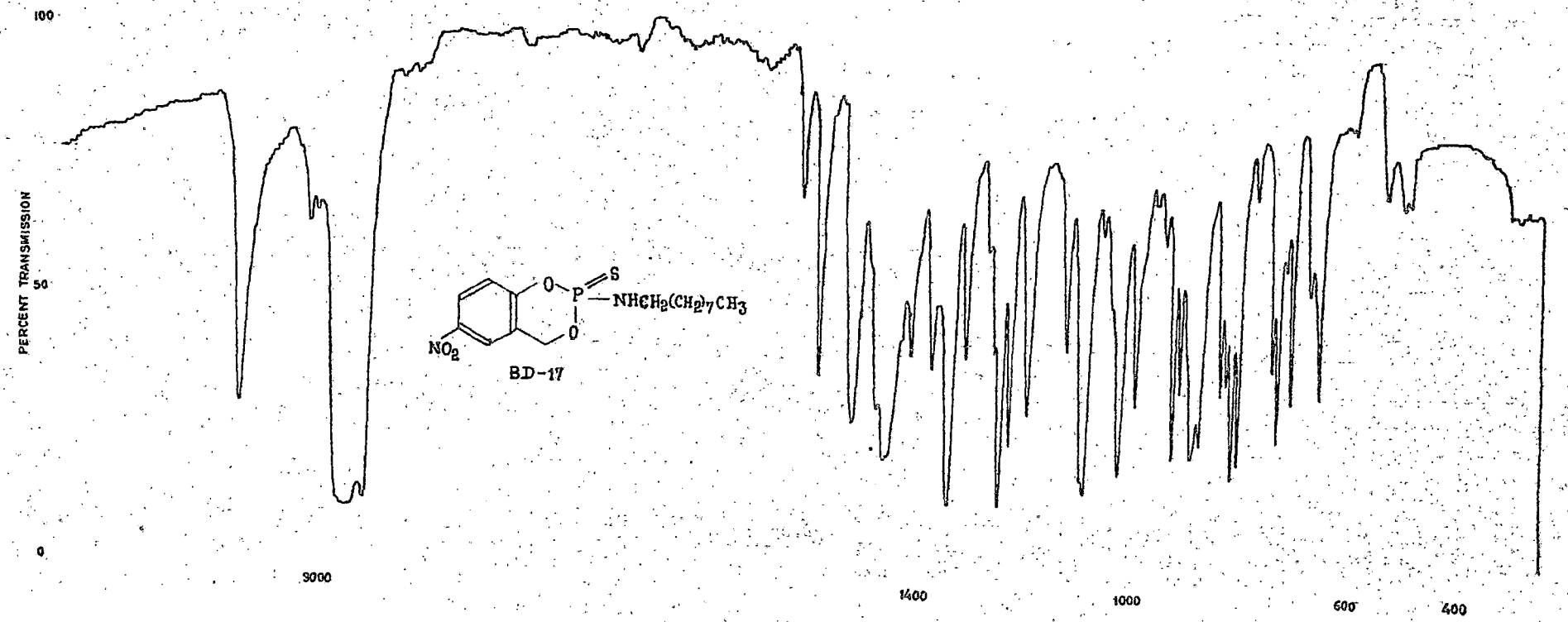
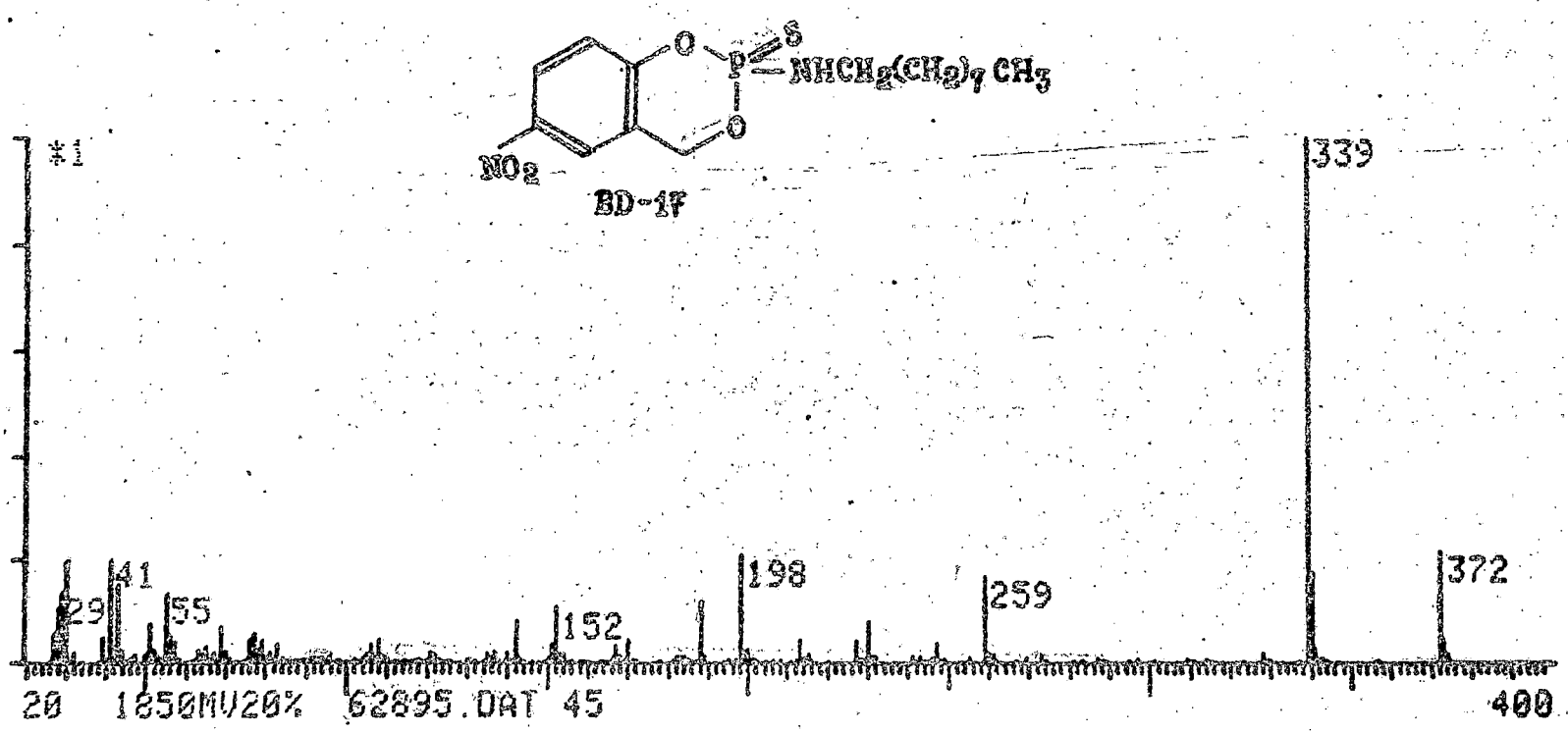


Fig. 7 IR Spectra of 2-Nonylamido-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-sulphide.

SPEKTRUM 45 VERDAMPFUNGSTEMPERATUR 140 GRAD
MOLEKUELPEAK: 372
MASSEN CHARAKTERISTISCHER IONEN:
339=372-SH

ANALYSE: 62895

STN HA 017 00
ST.STEENKEN
MESSG:
AUSH :25-MAR-81
AUSWER: SCH



BD-1F

Fig. 8 Mass Spectra of 2-Nonylamido-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-sulphide.

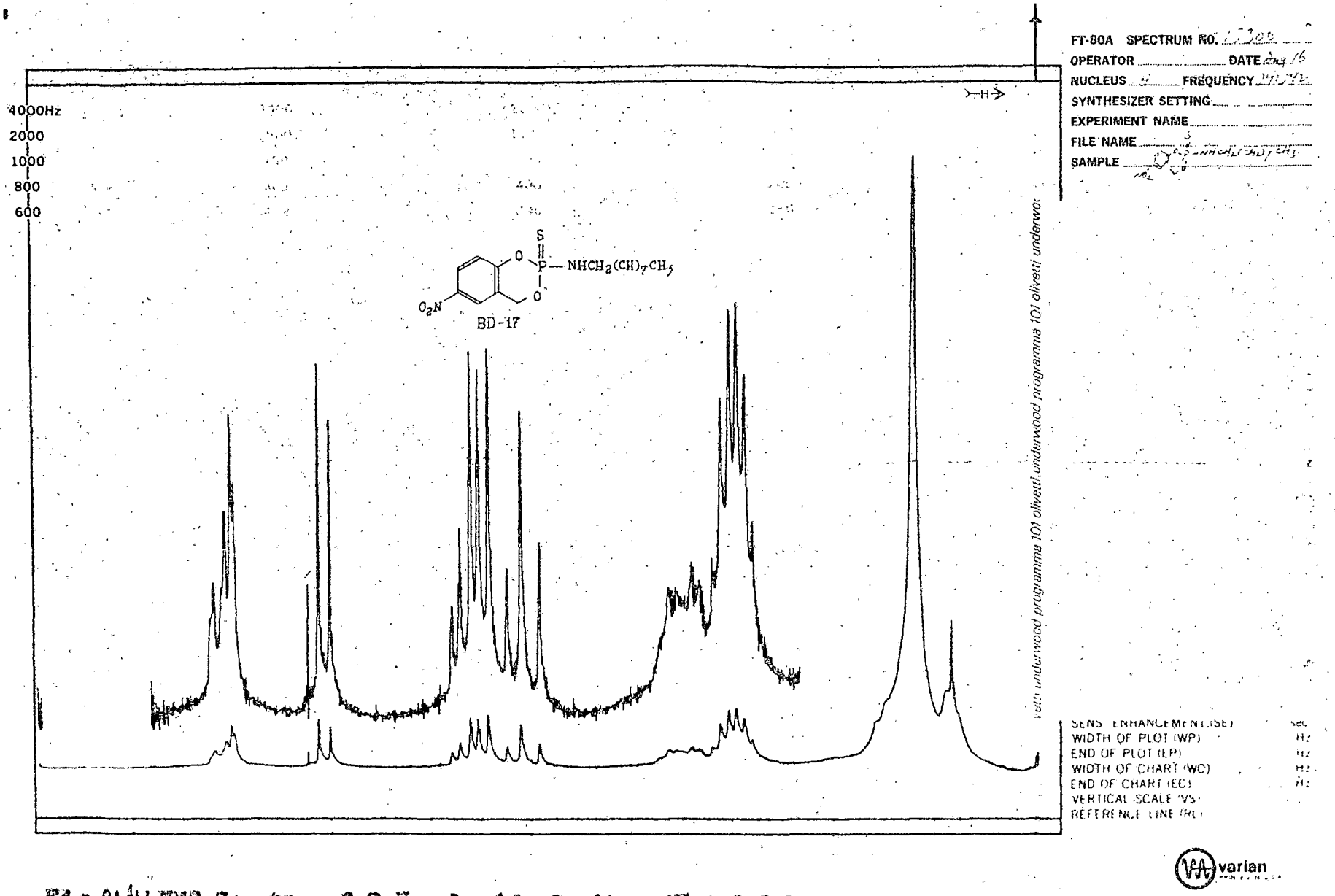
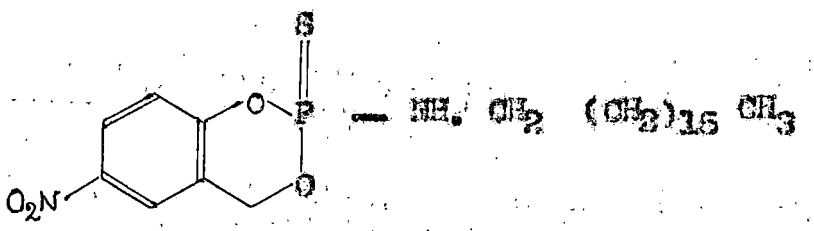


Fig.8A. ^{1}H NMR Spectra of 2-Nonylamido-6-nitro-4H-1,3,2-benzodioxaphosphrin-2-sulphide.

(iv) 2 - Heptadecylamido-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-sulphide (BD-18):



IR (Fig.9). (cm^{-1}):

660 (m) , 740 (m) , 810 (s) , 875 (s), 1030 , 1245 (s), 1345 (s), 1515 (s) , 1590 (m) , 1620 (w) and 3300 (s),

Mass (Fig - 10):

m/e,	484 (M^+) ,	451 ,	300 ,	259 ,	230 ,	198 ,	152
%I,	27.1 ,	100 ,	8.6 ,	29.3 ,	9.0 ,	28.6,	17.1

^1H NMR δ ($CDCl_3$) ppm (Fig-10A): 1.45, 3.15 , 3.3 – 3.8, 5.5, 7.05, 8.0, 8.15

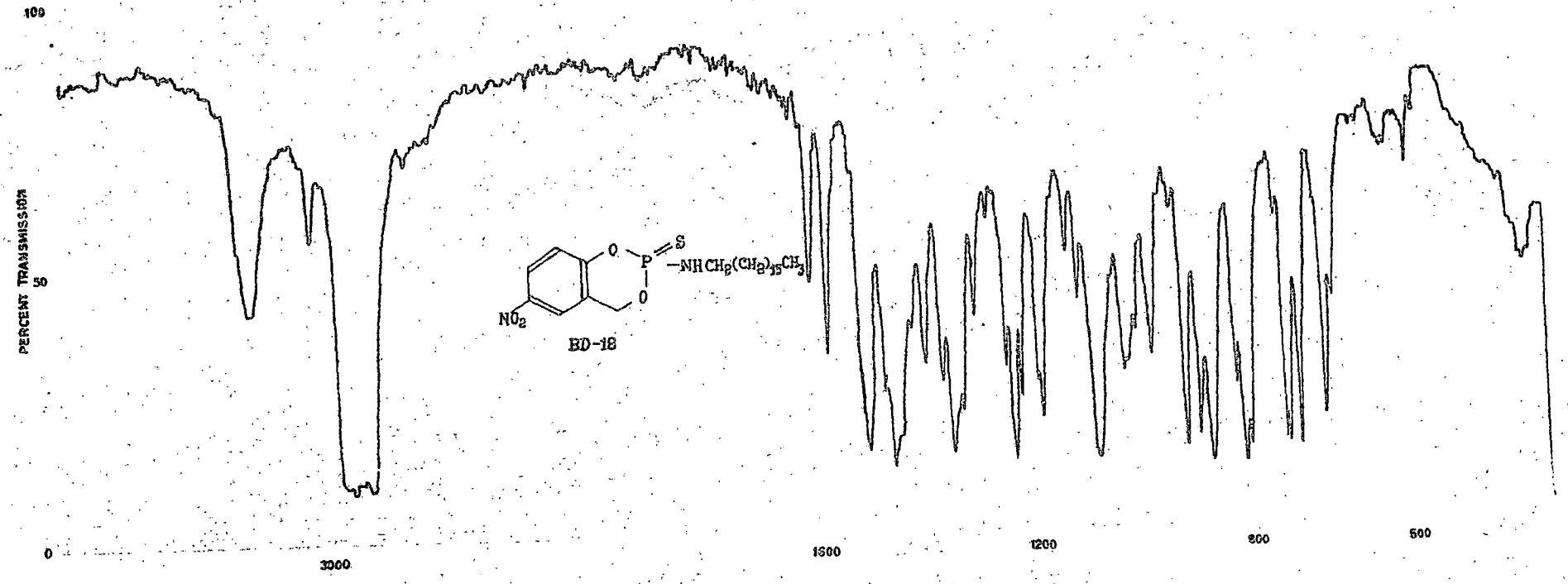
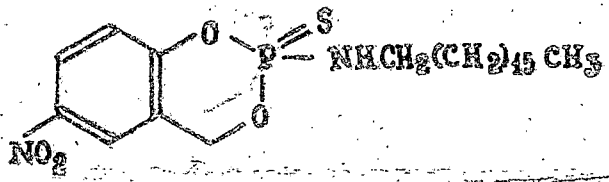


Fig. 9. IR Spectra of 2-Heptadecylamido-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-sulphide.

SPEKTRUM 45 VERDAMPFUNGSTEMPERATUR 170 GRAD
MOLEKUELPEAK: 484
MASSEN CHARAKTERISTISCHER IONEN:
451=484-SH

ANALYSE: 62892

STN HA 018 00
ST STEENKEN
MESSG:
AUSH :25-MAR-81
AUSWER: SCH



BD-18

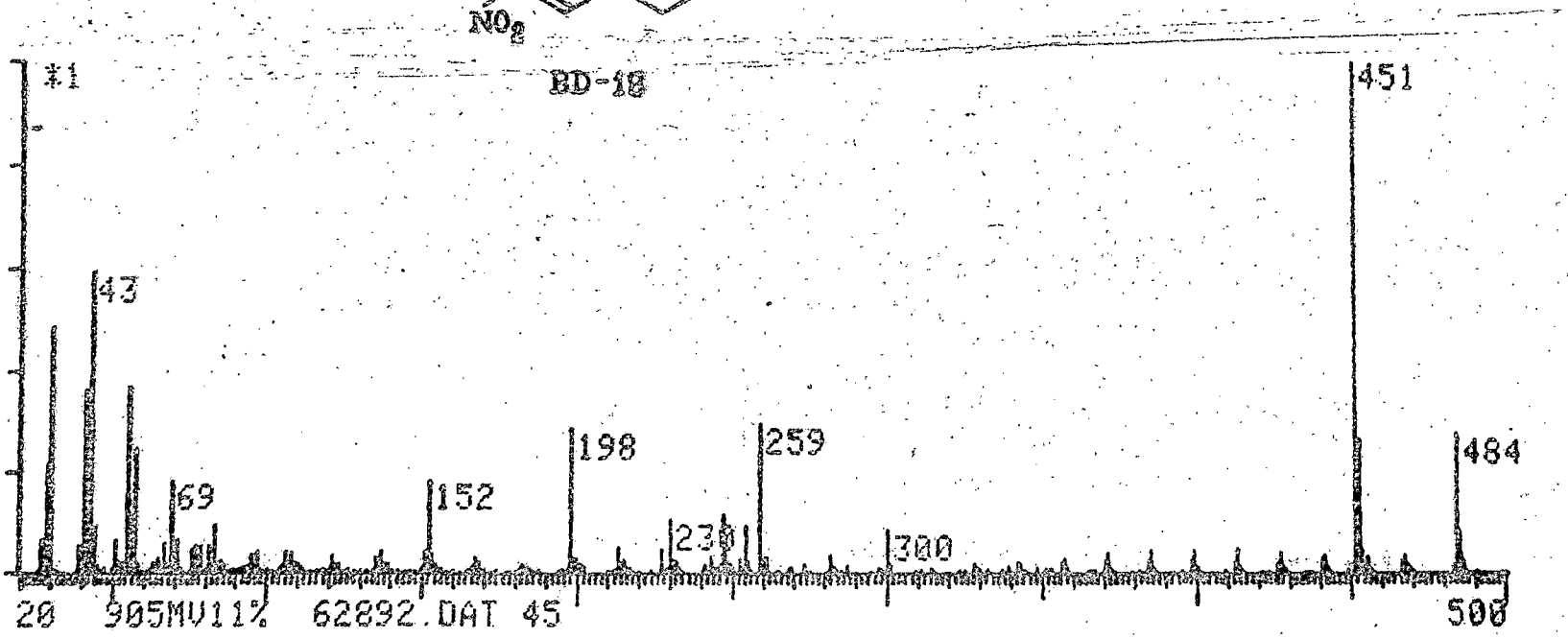


Fig.10 Mass Spectra of 2-Heptadecylamido-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-sulphide.

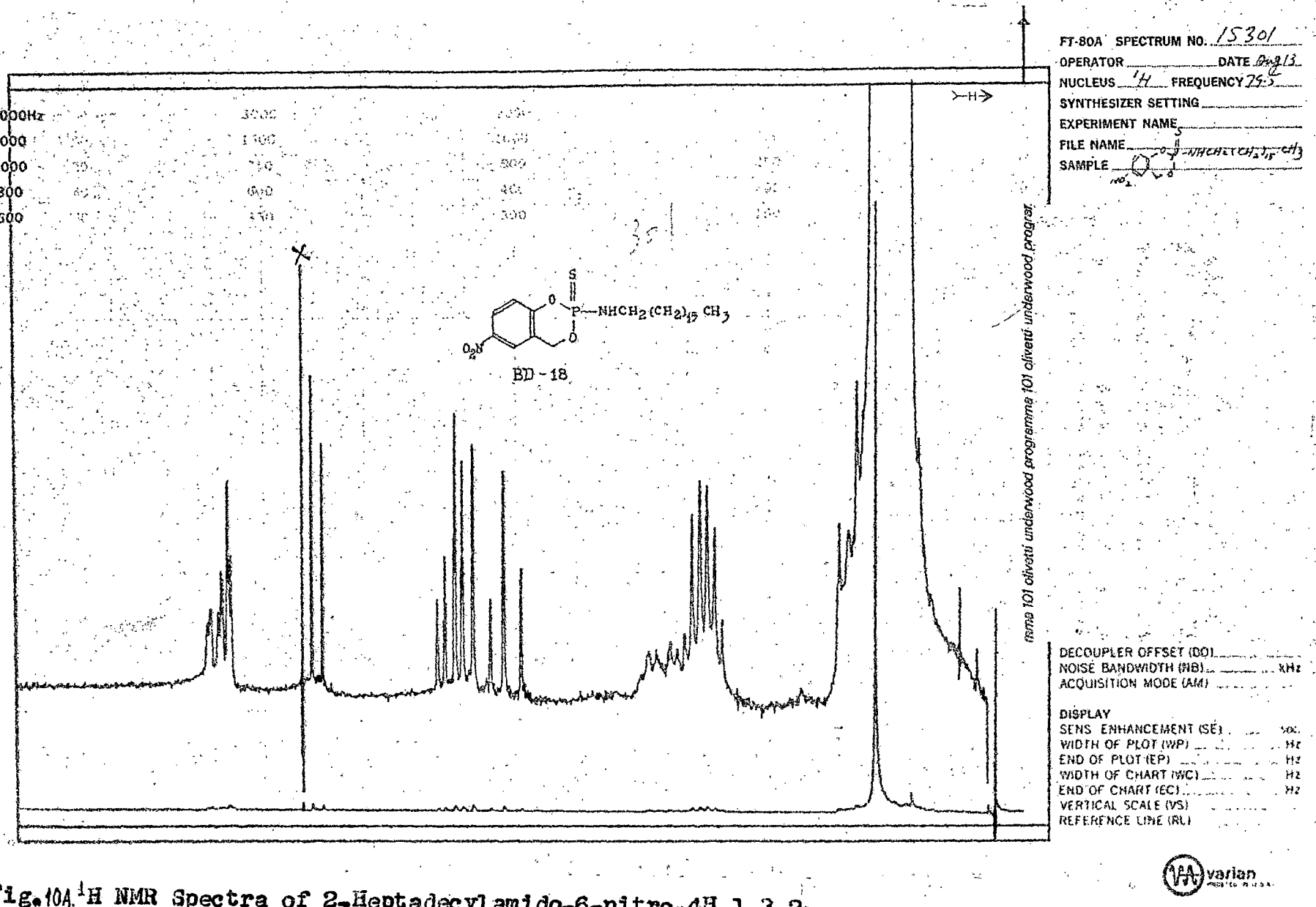


Fig.10A. ^1H NMR Spectra of 2-Heptadecylamido-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-sulphide.

2.(b) DISCUSSION ON SPECTRAL DATA:

The IR spectra of the compounds have been analysed according to Thomas[15]. The common IR bands for the compounds are summarized below:

1010 - 1030 cm^{-1} (s), P - O - C (alkyl);
1235 - 1250 cm^{-1} (vs), P - O - C (aryl);
880 - 920 cm^{-1} (s), P - O - C (aryl);
1515 - 1520 cm^{-1} (s), asym. str. of nitro group;
1340 - 1345 cm^{-1} (s), sym. str. of nitro group;
800 - 820 cm^{-1}, P = S (I);
640 - 660 cm^{-1}, P = S (II);

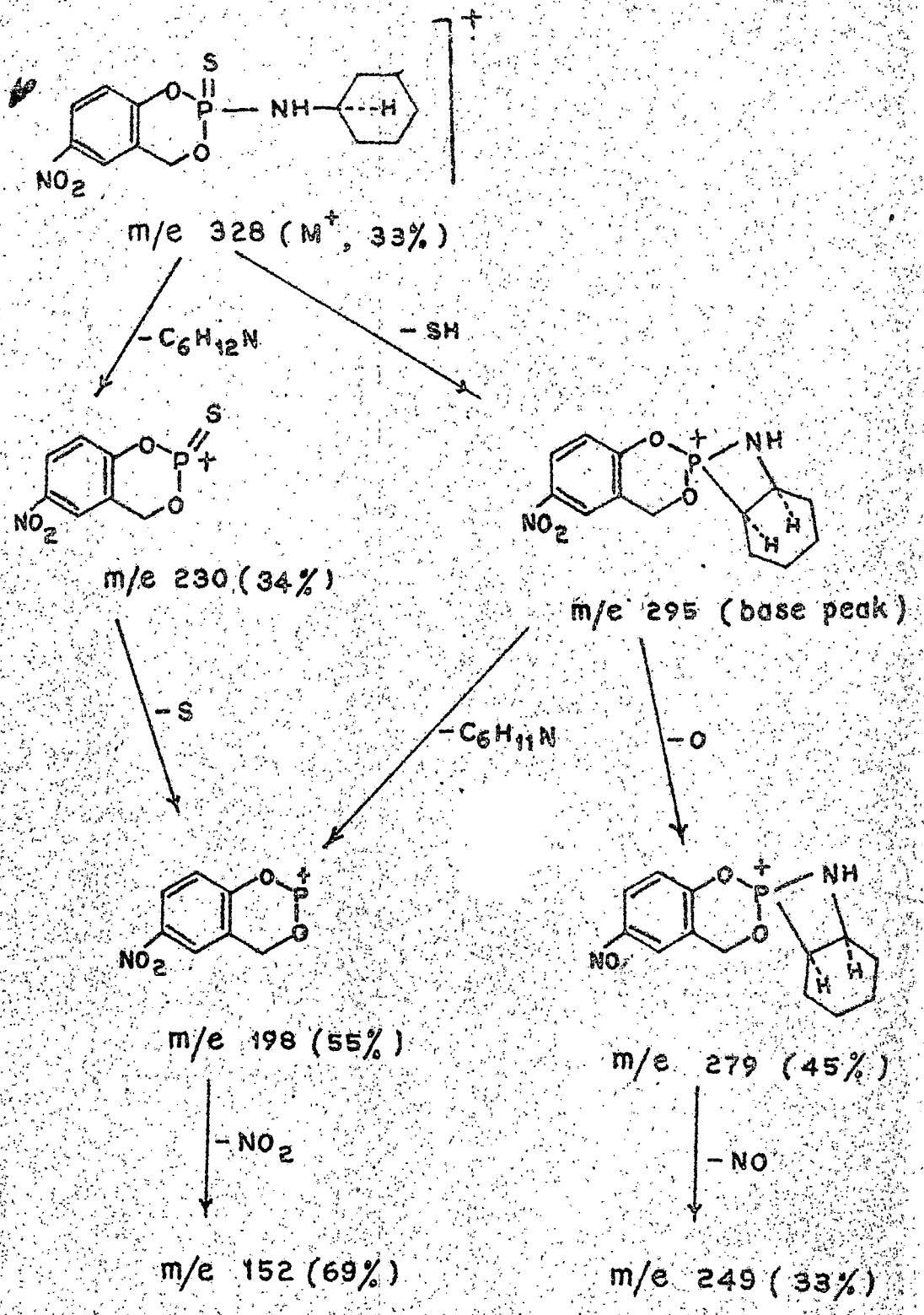
The thiono group is characterized by two IR absorption bands with frequencies in the normal ranges given by Thomas[15], as both are not observed in amidophosphates; of these two, the lower frequency band is assigned to P = S (II) bond stretching vibration frequency. The origin of the higher frequency band P = S (I) is uncertain, but whatever its origin, its diagnostic value is beyond doubt. It is also observed that neither of two bands shows any systematic shifts which reflects changes in the inductive properties of the substituent; this is not unexpected if they do indeed arise from mixed modes. The P - O - C (alkyl) group is characterized by a strong absorption band whose frequency lies between 1010 - 1030 cm^{-1}. While

the band due to P - O - C (aryl) group is found in the 1235 - 1250 cm^{-1} region; this band is always accompanied by a second absorption band in the 880 - 920 cm^{-1} region. Two bands present in the ranges 1515 - 1520 cm^{-1} and 1340 - 1345 cm^{-1} are due to asym. and sym. stretching of nitro group respectively. The bands present at 1620 cm^{-1} and 1585 cm^{-1} are due to "quardrant stretching" C = C vibration of the aromatic ring.

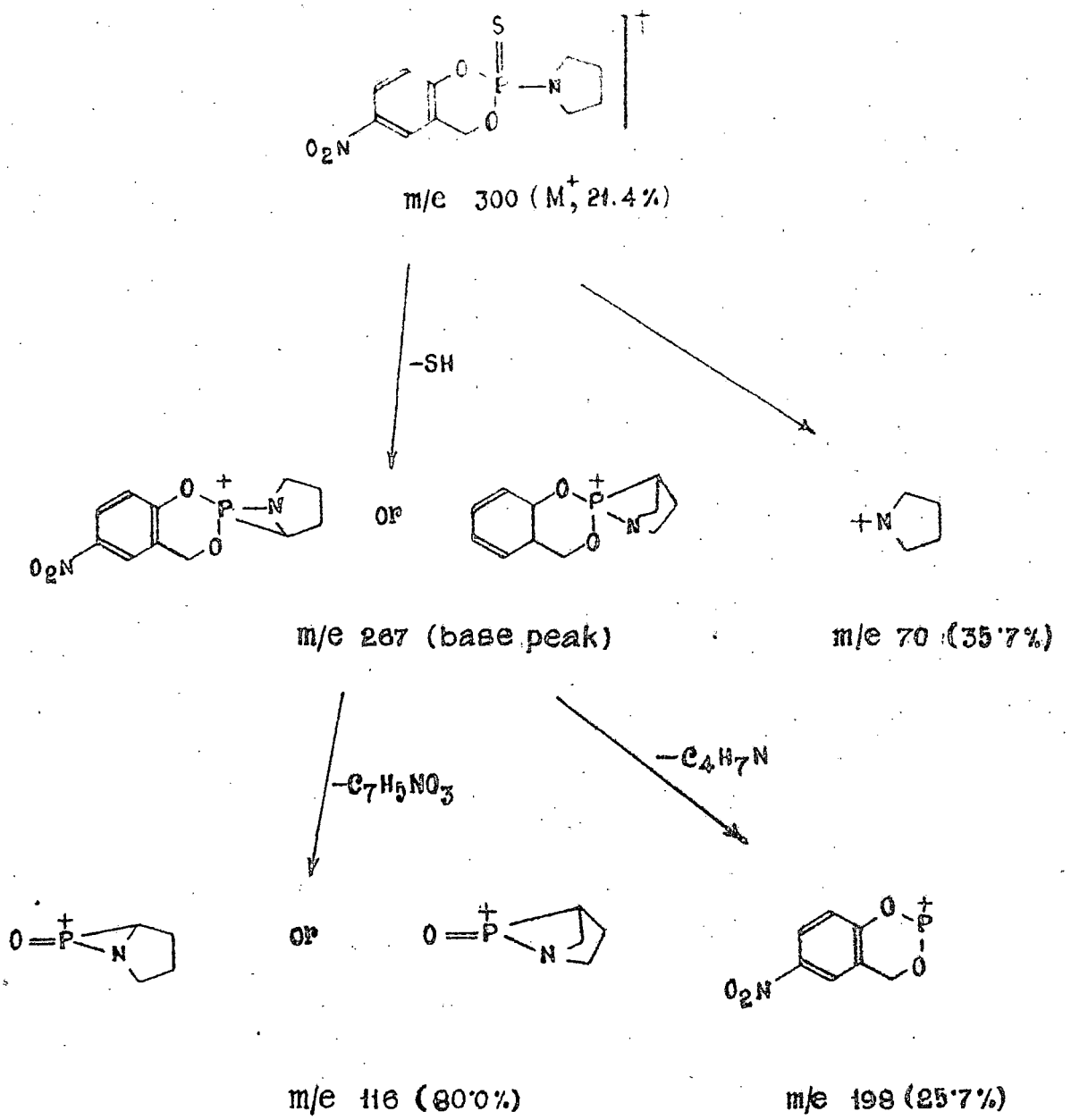
The mass spectra of these compounds have been analysed according to cooks and Gerrard[16]. The alkylamidophosphorothionates show parent molecular ion peaks. Fragmentation by loss of 'SH' radical is important; all compounds (data for only four compounds have been presented) show an ion due to $(M - SH)^+$, and it is the base peak for all alkylamidophosphorothionates.

BD-10 shows m/e 295 ion as the base peak by the direct elimination of SH from the molecular ion peak (m/e 328, %RI 33, Scheme-I). The ion (m/e 230, %RI 34) is formed by the direct loss of $C_6H_{12}N$ from the molecular ion. The ion (m/e 198, %RI 55) is formed by loss of 'S' from the ion m/e 230 and also by the elimination of $C_6H_{11}N$ from the base peak ion. The ion (m/e 152, %RI 69) is formed by the elimination of NO_2 from the ion m/e 198. The peak for m/e 279, %RI 45 is observed due to the elimination of 'O' from the base peak, and then the ion (m/e 249, %RI 33) is formed by loss of NO from the ion, m/e 279.

BD-15 shows m/e 267 ion as the base peak by the direct elimination of SH from the M^+ ion peak (Scheme-II). The ion (m/e 116,

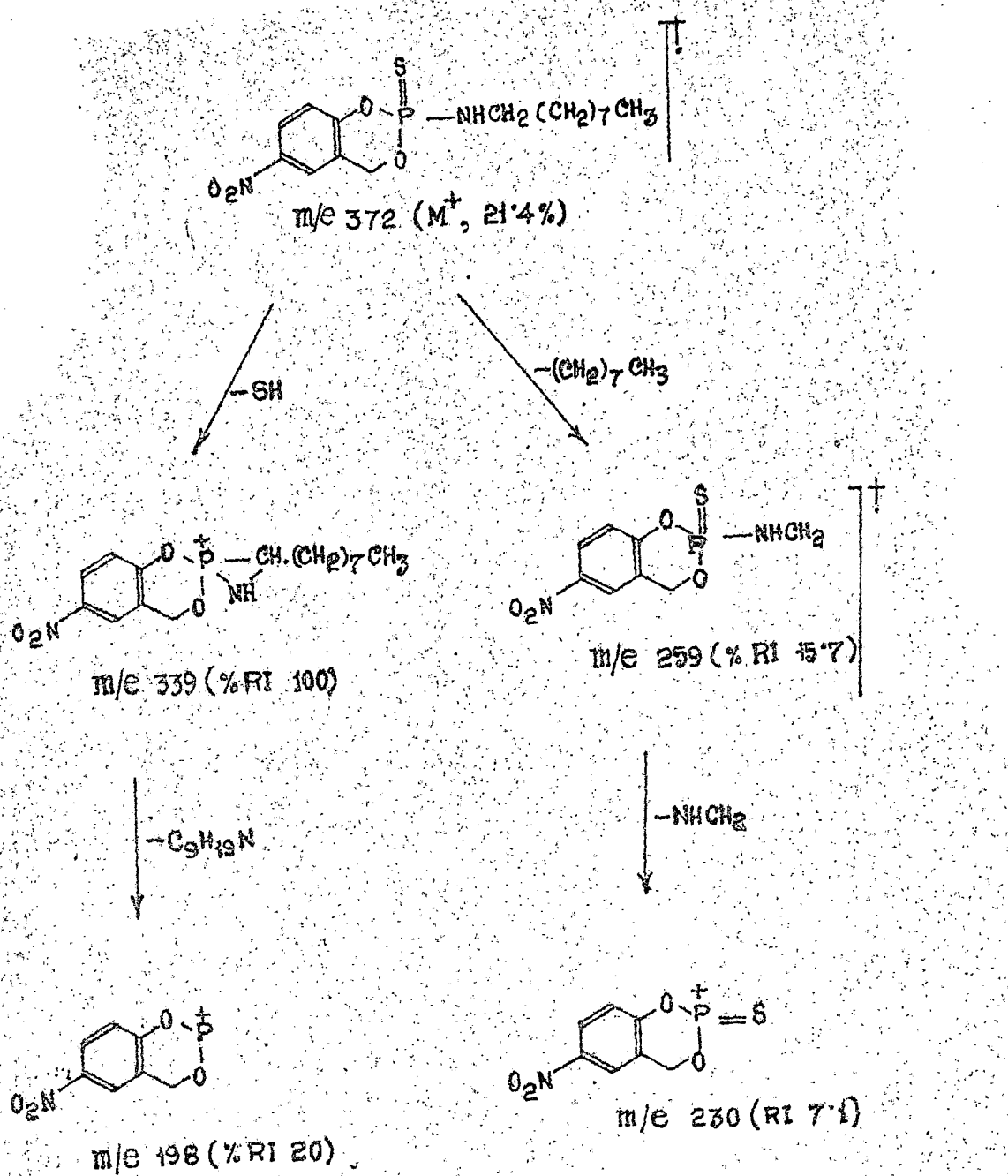


Scheme. I Mass fragmentation of BD_{10}



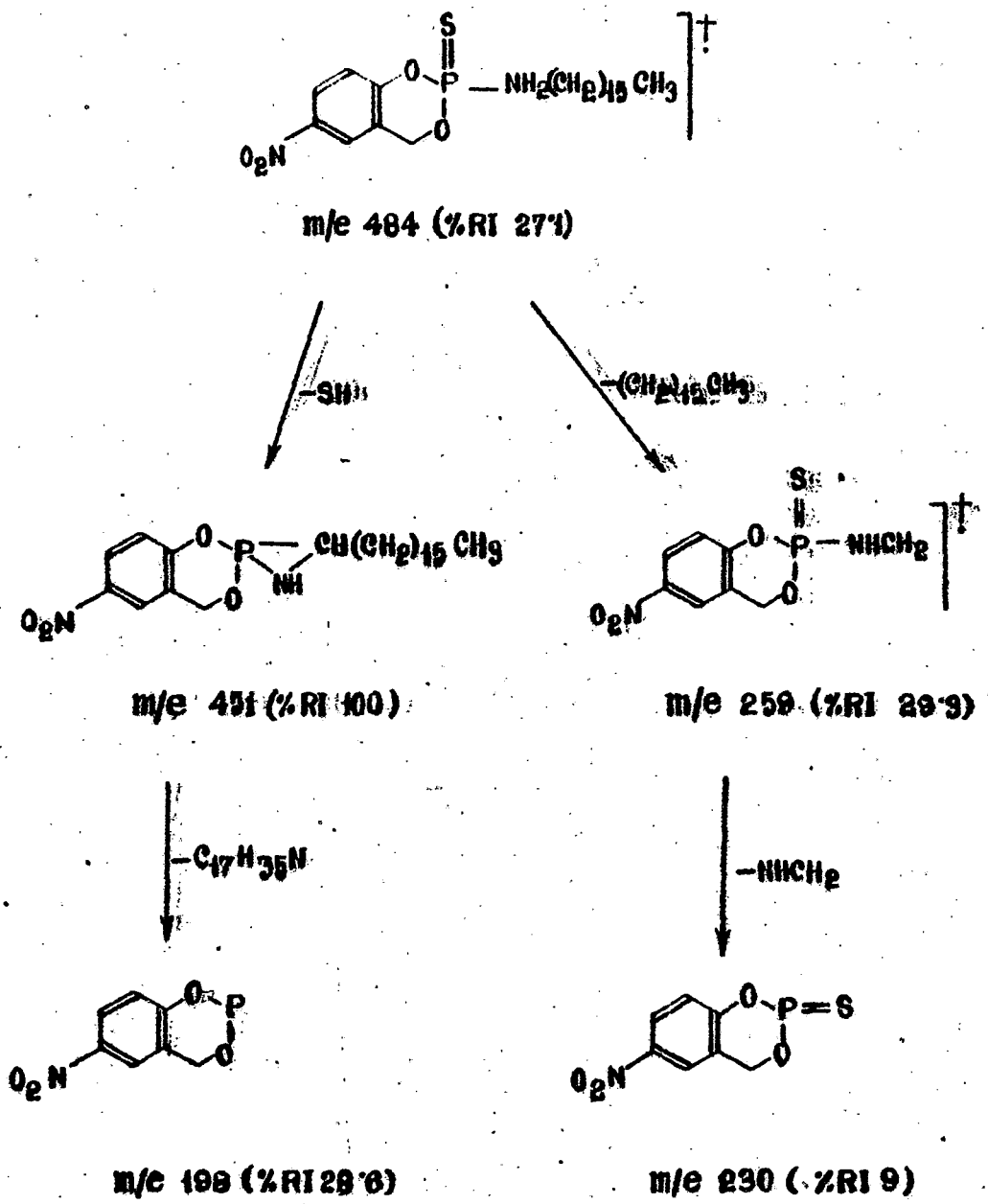
Scheme II

Mass fragmentation processes in BD-15



Scheme III

Mass fragmentation of BD-17



Scheme IV

Mass fragmentation processes in BD-18

% RI 80) is formed by the direct loss of $C_7H_5NO_3$ from the base peak ion; the ion (m/e 198, % RI 25.7) is also formed by the direct loss of C_4H_7N from the base peak ion.

BD-17 shows m/e 339 ion as the base peak by the direct elimination of SH from the parent molecular ion (m/e 372, % RI 21.4, scheme III): The ion (m/e 259, % RI 15.7) is formed by the direct loss of C_8H_{17} from the M^+ ion peak; the ion (m/e 198, % RI 20) is formed by the loss of $C_9H_{19}N$ from the base peak ion. The ion (m/e 230, % RI 7.1) is formed by the loss of CH_3N from the ion m/e 259.

BD-18 shows m/e 451 ion as the base peak by the direct elimination of SH from the molecular ion peak (m/e 484, % RI 27.1) (scheme -IV). The ion (m/e 198, % RI 28.6) is formed by the loss of $C_{17}H_{35}N$. from the base peak ion. The ion (m/e 250, % RI 29.3) is formed by the elimination of $-(CH_2)_{15}.CH_3$ from the molecular ion, and the ion (m/e 230, % RI 9) is formed by the elimination of $-NH.CH_2$ from the ion m/e 259 (scheme-IV).

Finally the structure of different compounds has been settled by taking ^1H NMR spectra, and analysis of spectral data has been given above [in 2(a)].

3. DISCUSSION ON FUNGICIDAL ACTIVITY (P. oryzae - Growth Inhibition):

The data (% inhibition) for in vitro growth inhibition studies against the blast disease of rice caused by P. oryzae are listed in Table - IA to IJ (Page-162 to 171). The ED_{50} values (μg/ml) calculated

by least - squares regression analysis are given in Table - 1.

The results reveal that all compounds show good inhibitory effect on the growth of P. oryzae. At 72 hours the ED_{50} value increases in the following order:

BD-16 < BD-11 < BD-10 < BD-12 < BD-17 < BD-15 < BD-14 < BD-18 < BD-13

i.e. the dimethylamido compound (BD-13) is most effective, and its inhibitory effect can be comparable with that of Hinosan. At 48 hours both the dimethylamido and the isopropylamido (BD-14) compounds are most active; other compounds are also effective, but their activities are less than that of Hinosan. The ED_{95} values ($\mu g/ml$) calculated from the least-square straight lines are presented in Table-2, and it can be observed that BD-13 and BD-14 are 2-times less active compared to Hinosan.

Table - 1

Fungicidal activity of different compounds against Pyricularia oryzae

Code name of the Compound	ED_{50} in $\mu g/ml$			
	48 hours	72 hours	96 hours	120 hours
BD - 10	12.30	30.19	47.86	66.06
BD - 11	16.59	30.90	38.90	61.65
BD - 12	10.47	19.49	25.11	35.48
BD - 13	<5.00	5.88	10.96	21.87
BD - 14	<5.00	>12.50 <25.00	>12.50 <25.00	>25.00 >50.00
BD - 15	10.71	14.12	17.37	31.62
BD - 16	23.44	38.01	45.70	61.65
BD - 17	11.22	18.19	25.11	40.73
BD - 18	9.12	11.22	27.54	38.01
Hinosan (Standard)	<5.00	<5.00	<5.00	<5.00

(Ref. Table -1A to 1J, page -162 to 171)

Table - 2

Fungicidal activity of different compounds against Pyricularia oryzae.

Code name of the Compound	ED_{95} in $\mu g/ml$			
	48 hours	72 hours	96 hours	120 hours
BD - 10	60.25	275.42	537.03	616.59
BD - 11	138.03	229.03	251.18	339.04
BD - 12	69.18	134.89	151.35	194.98
BD - 13	>12.50 <25.00	25.70	48.97	134.89
BD - 14	>5.00 <12.50	>25.00 <50.00	>25.00 <50.00	>50.00 <100.00
BD - 15	58.88	91.20	93.32	169.82
BD - 16	154.88	275.42	281.83	363.07
BD - 17	63.09	123.02	158.48	194.98
BD - 18	45.70	138.03	147.91	177.82
Hinosan (Standard)	>5.00 <12.50	>12.50 <25.00	>25.00 <50.00	>25.00 <50.00

[Ref. Tables - 1A to 1J, page -162 to 171]

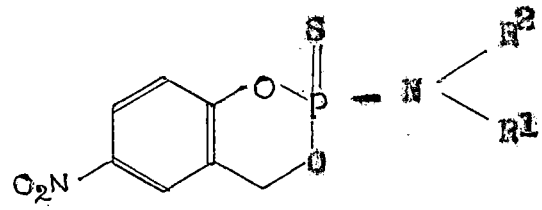
4. DISCUSSION ON INSECTICIDAL ACTIVITY:

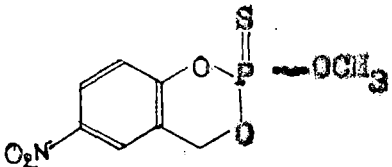
The insecticidal activity data of the compounds against Grasshoppers, Oxya nitidula are listed in Table - 3, and the results have been compared with that of salithion and the 2-methoxy - 6 - nitro-4H - 1,3,2 - benzodioxaphosphorin-2- sulphide (BD-8).

The data presented in Table - 3 reveal that all alkylamidophosphorothionates have less insecticidal activity than the methoxy compound (BD-8) and Salithion. The dimethylamido (BD-13) and the morpholino (BD-11) compounds have some insecticidal activity, but the other compounds are non-insecticidal. In whole series of nitro-saligenin cyclic alkyl/phenyl/alkylamidophosphorothionates synthesized in our Laboratory, only the methoxy compound (BD-8) shows insecticidal activity comparable with that of Salithion (17).

Table - 3

Insecticidal Activity on Grasshoppers (Oxya nitidula)



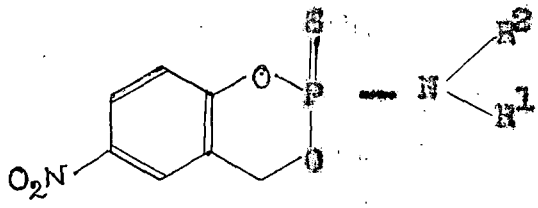
Amido Group	Code No.	Minimum Conc. (range) for 100 percent mortality (LC_{100}, $\mu g/gm$ range).	Average LC_{100} value ($\mu g/gm$)
Cyclohexylamido	BD - 10	>10	>10 (0%)
Morpholino	BD - 11	6 - 7	6.5
Dimethylamido	BD - 13	3 - 4	3.5
Isopropylamido	BD - 14	>10	>10 (0%)
Pyrrolidino	BD - 15	>10	>10 (0%)
Piperidino	BD - 16	>10	>10 (0%)
Nonylamido	BD - 17	>10	>10 (0%)
Heptadecylamido	BD - 18	>10	>10 (0%)
	BD - 8	<0.5	<0.5
Salithion	---	<0.5	<0.5

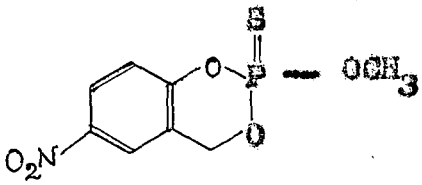
5. DISCUSSION ON ACUTE ORAL TOXICITY ON RAT:

The acute oral toxicity data (LD_{50}) of the compounds (BD-10 to BD-15) on male rats are presented in Table-4, and the results have been compared with salithion and with the methoxy compound (BD-8). All compounds (BD-10 to BD-15) are less toxic than salithion. BD-12, BD-13, BD-15 and BD-8 have greater toxicity to rats compared to other nitro saligenin cyclic phosphoramidothionates. 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. The decrease of spontaneous motor activity was occured after 2 & 4 hours for all compounds except BD-15; in BD-15 it appeared after 60 - 72 hours.

Table - 4

LD_{50} value of different compounds in Rats.



Amido Group	Code No.	LD_{50} (mg/kg)
Cyclohexylamido	BD - 10	>250
Morpholino	BD - 11	>250
Diethylamido	BD - 12	150 - 250
Dimethylamido	BD - 13	125 - 250
Isopropylamido	BD - 14	>250
Pyrrolidino	BD - 16	150 - 250
	BD - 8	120 - 135
Salithion	--	102

6. DISCUSSION ON PHYTOTOXICITY (against wheat seed germination):

The effects of the alkylamidophosphorothionates on the germination of wheat seeds (Triticum Spp, U.P. 262 variety) are summarised in Table - 5. The results indicate that none of the compounds are phytotoxic upto 600 ppm concentration; however, BD-10, BD-11 and BD-17 show 90 percent germination at 500 ppm.

Table - 5

The effects of Alkylamidophosphorothionates on germination of wheat seed (Triticum Spp)

Code No.	Percent germination at different concentration		
	500 ppm	250 ppm	100 ppm
BD - 10	90	100	100
BD - 11	90	100	100
BD - 12	100	100	100
BD - 13	100	100	100
BD - 14	100	100	100
BD - 15	100	100	100
BD - 16	100	100	100
BD - 17	90	100	100
BD - 18	100	100	100

7. DISCUSSION ON ANTICHOLINESTERASE ACTIVITY:

The acetylcholinesterase inhibition data for housefly head homogenate (HFAChE) and goat whole blood (blood - ChE) are listed in Table 6A - 6J (page -172 to 181); the molar I_{50} values calculated by least square programme are given below (Table - 6). The data for only five compounds have so far been taken.

Table - 6

Anticholinesterase activity on housefly head homogenate and goat whole blood.

Code No.	I_{50} (M) x 10^{6} (HFAChE) housefly.	I_{50} (M) x 10^{4} (ChE) Goat whole blood
BD - 10	2.86	1.81
BD - 11	5.43	1.04
BD - 12	8.26	1.43
BD - 13	1.80	4.66
BD - 14	1.86	4.78

It has been observed that for any alkylamidophosphorothionates (BD-10 to BD-14), the housefly head acetylcholinesterase is more inhibited than the blood cholinesterase. For the housefly head acetylcholinesterase, the I_{50} value increases in the following order:

$$BD\text{-}13 < BD\text{-}14 < BD\text{-}10 < BD\text{-}11 < BD\text{-}12$$

i.e., the antiacetylcholinesterase activity of the dimethylamido compound is highest, and that of the diethylamido-phosphorothionate is least. For the blood cholinesterase, the I_{50} value increases in the following order:

$$BD\text{-}11 < BD\text{-}12 < BD\text{-}10 < BD\text{-}13 < BD\text{-}14$$

i.e. the anticholinesterase activity of the morpholino compound is most and that of the isopropylamidophosphorothionates is least.

8. DISCUSSION ON CHEMICAL HYDROLYSIS:

The chemical hydrolysis of some of the compounds have been carried out in 0.0095 M NaOH (pH 11.95) at 410 nm; three sets of experiments in each case have been performed. The hydrolysis data for each compound at 410 nm along with the regression co-efficient value, and the value of K_{hyd} calculated thereof for a particular set are given in Table - 7A to 7E (page-182 to 186). The data have been summarized in the following table (Table - 7):

Table - 7

Hydrolysis of nitro saliganin cyclic alkylamidophosphorothionates (pH 11.85, temp. 20°C, solvent 50% ethanol).

Code No.	K_{hyd} (min^{-1})	Ref. Table No.	Average K_{hyd} (min^{-1})
BD - 11	6.1413×10^{-5}	7A (page 182)	6.142×10^{-5}
BD - 13	2.687×10^{-5}	7B (page 183)	2.88×10^{-5}
BD - 15	1.7734×10^{-5}	7C (page 184)	1.763×10^{-5}
BD - 16	1.2129×10^{-5}	7D (page 185)	1.162×10^{-5}
BD - 17	20.366×10^{-3}	7E (page 186)	19.40×10^{-3}

The average hydrolysis constant recorded in Table - 7 show that the nature of the amido group in the exocyclic side chain influences the stability of the compounds to alkaline hydrolysis. The K_{hyd} increases in the following order:

$$BD - 16 < BD - 15 < BD - 13 < BD - 11 < BD - 17$$

i.e., the Piperidino compound (BD - 16) is most stable, and the nonylamido compound (BD - 17) is least stable to alkaline hydrolysis. The cyclic alkylphosphoramidothionates containing the disubstituted amido groups are extremely resistant to hydrolysis compared to the other compound having the monosubstituted amido group (nonylamido); probably the

the steric-interferences of the nonylamido group are less compared to that of the piperidino, pyrrolidino, morpholino and dimethylamido groups.

Contrary to alkaline hydrolysis in 0.0095 M NaOH these alkylamidophosphorothionates show a good deal of registance to the hydrolysis at pH 7.7 in phosphate buffer. For example no detectable hydrolysis occurs even after thirtysix hours in case of dimethylamido, piperidino, pyrrolidino and morpholino compounds. However, the nonylamido compound shows slight hydrolysis at pH 7.7 in phosphate buffer; the rate of hydrolysis at pH 7.7 is less than that at pH 11.85. It may concluded that the rate of alkaline hydrolysis is increased as the pH value increases from 7.7 to 11.8.

From the above studies it is clear that these alkylamidophosphorothionates are almost non-insecticidal; however, they have good fungicidal activities, and have no phytotoxic properties. For these reasons further studies on fungicidal activities (both *in vivo* & in vitro) against some other pathogenic fungi, and also phytotoxic properties have been performed.

9. DISCUSSION OF FUNGICIDAL ACTIVITY ON OTHER FUNGI (Growth inhibition).

The data (% inhibition) for *in vitro* studies against *V. albo-atrum*, *A. solani* and *H. oryzae* are listed in Tables - 8A to 8J. (page- 187 to 196), 9A to 9I (page- 197 to 205) and 10A to 10I (page- 206 to 214) respectively. The ED_{50} and ED_{95} values (μ g/ml) calculated by least square regression method are given in Tables - 8 to 10.

The results reveal that all compounds show inhibitory effect on the growth of *V. albo-atrum*, *A. solani* and *H. oryzae*. For *V. albo-atrum* at 48 hours the ED_{50} value increases in the following order:

BD-18 < BD-12 < BD-17 < BD-15 < BD-16 < BD-14 < BD-13 < BD-11 < BD-10

i.e., the cyclohexylamido compound (BD-10) is most effective, but its inhibitory effect is about 1.5 times less than that of Hinosan.

For *A. solani* at 72 hours the ED_{50} values increases in the following order:

BD-17 < BD-13 < BD-11 < BD-18 < BD-14 < BD-12 < BD-16 < BD-10.

i.e., the compound (BD-10) is most effective and its inhibitory effect can be comparable with that of Hinosan. The inhibitory effects of the compounds BD-12, BD-15 and BD-16 are nearly 1.5 times less than that of Hinosan.

For *H. oryzae* at 72 hours the ED_{50} values increases in the following order:

BD-11 < BD-12 < BD-13 < BD-17 < BD-10 < BD-15 < BD-16 < BD-14

i.e., the isopropylamido compound (BD-14) is most effective, and its inhibitory effect is nearly 1.6 times less than that of Hinosan.

To sum up, the dimethylamido compound is most effective against *P. oryzae*; the cyclohexylamido compound is most active against *V. albo-atrum* and *A. solani*; and the isopropylamido compound is most effective against *H. oryzae*. But all of these compounds show less inhibitory effects than that of Hinosan.

Table - 8(1)

Fungicidal activity of different compounds against Verticillium albo-atrum

Code name of the Compound.	ED_{50} in μg/ml		
	24 hours	48 hours	62 hours
BD - 10	91.20	139.03	154.88
BD - 11	70.79	141.26	190.54
BD - 12	144.54	190.54	234.42
BD - 13	104.71	161.35	181.97
BD - 14	104.71	151.83	199.52
BD - 15	95.49	169.82	181.97
BD - 16	97.72	158.48	190.54
BD - 17	138.03	173.78	234.42
BD - 18	162.18	194.98	229.08
Hinosan (Standard)	>50.00	95.49	123.03

[Ref. Tables 8A to 'J, page - 187 to 196]

Table - 8 (2)

Fungicidal activity of different compounds against Verticillium albo-atrum.

Code name of the Compound	ED_{95} in μg/ml		
	24 hours	48 hours	62 hours
BD - 10	316.22	501.18	549.54
BD - 11	213.79	457.08	538.84
BD - 12	398.10	588.84	707.94
BD - 13	323.59	467.73	575.43
BD - 14	354.81	501.18	588.84
BD - 15	380.18	537.03	602.55
BD - 16	288.40	467.73	594.54
BD - 17	467.73	588.84	630.95
BD - 18	676.08	758.57	851.13
Hinosan (Standard)	>200·00	295.12	346.73

[Ref. Tables 8A to 8J, page - 187 to 196]

Table - 9 (1)

Fungicidal activity of different compounds against Alternaria solani.

Code name of the Compound	ED_{50} in μ g/ml			
	24 hours	48 hours	72 hours	96 hours
BD - 10	>50.00	75.85	120.22	147.91
BD - 11	<100.00	147.91	208.92	245.47
BD - 12	93.32	125.89	144.54	194.98
BD - 13	>50.00	134.89	229.08	281.83
BD - 15	<50.00	114.81	158.48	208.92
BD - 16	<50.00	89.12	128.82	169.92
BD - 17	100.00	173.78	257.03	288.40
BD - 18	50.00	77.62	136.20	239.88
Hinosan (Standard)	<50.00	60.25	104.71	169.82

[Ref. Tables 9A to 9I, page - 197 to 205]

Table - 9 (2)

Fungicidal activity of different compounds against Alternaria solani

Code name of the Compound	ED_{95} in μ g/ml			
	24 hours	48 hours	72 hours	96 hours
BD - 10	>150.00	288.40	446.68	588.84
BD - 11	>150.00	575.43	616.59	676.08
BD - 12	309.02	363.07	436.51	549.54
BD - 13	>100.00	426.57	741.31	1000.00
BD - 15	>100.00	363.07	575.43	645.65
BD - 16	>100.00	323.59	426.57	575.43
BD - 17	>150.00	512.86	776.24	891.25
BD - 18	>150.00	245.47	588.84	776.24
Hinosan (Standard)	>100.00	177.82	309.02	446.68

[Ref. Tables 84 to 91, page - 197 to 205]

Table - 10 (I)

Fungicidal effect of different compounds against Helminthosporium oryzae.

Code name of the Compound	ED_{50} in μg/ml			
	24 hours	48 hours	72 hours	96 hours
BD - 10	<50.00	125.89	181.97	194.98
BD - 11	123.02	269.15	338.84	389.04
BD - 13	>100.00	223.87	263.02	316.22
BD - 14	<50.00	83.17	131.82	186.20
BD - 15	>50.00	154.88	173.78	208.92
BD - 16	<50.00	114.81	158.48	199.52
BD - 17	66.06	186.20	229.08	257.03
BD - 18	194.98	251.18	316.22	363.07
Hinosan (Standard)	39.81	63.09	81.28	93.32

(Ref. Tables 10A to 10I, page- 206 to 214)

Table - 10 (2)

Fungicidal effect of different compounds against Helminthosporium oryzae.

Code name of the Compound.	ED_{95} in μ g/ml			
	24 hours	48 hours	72 hours	96 hours
BD - 10	>100.00	500.00	575.43	602.55
BD - 11	562.34	977.23	1122.01	1548.81
BD - 13	>150.00	724.43	851.13	954.99
BD - 14	>100.00	263.02	407.38	630.95
BD - 15	>150.00	500.00	524.80	616.59
BD - 16	>100.00	549.54	602.55	758.57
BD - 17	346.73	524.80	602.55	707.94
BD - 18	524.80	707.94	891.25	1023.29
Hinosan (Standard)	186.20	218.77	269.15	309.02

[Ref. Tables 10A to 10I, page - 206 to 214]

10. DISCUSSION ON FUNGICIDAL ACTIVITY (Spore germination).

The data (% inhibition) for *in vitro* spore germination inhibition against *A. niger* & *P. gensenl* (Store Fungi), and *H. oryzae* & *V. albo-atrum* are listed in Tables - 11A to 11I (page - 215 to 223), 12A to 12I (page - 224 to 232), 13A to 13I (page - 233 to 241) and 14A to 14I (page - 242 to 250) respectively. The ED_{50} and ED_{95} values are given in Tables 11 to 14.

The results reveal that all compounds are effective on spore germination inhibition of *A. niger*; *P. gensenl*; *H. oryzae* and *V. albo-atrum*.

For *A. niger* at 48 hours the ED_{50} value increases in the following order:

BD-16 < Hinosan < BD-10 < BD-11 < BD-18 < BD-13 = BD-15 < BD-14 < BD-17

i.e., the inhibitory effects of all compounds except BD-16; are greater than that of Hinosan; the nonylamido compound (BD-17) is the most effective one.

For *P. gensenl* at 48 hours the ED_{50} value increases in the following order:

BD-16 < BD-14 < BD-11 < BD-18 < BD-10 < Hinosan < BD-15=BD-17 < BD-13

i.e., the dimethylamido compound (BD-13) is most effective, and its inhibitory effect is about 1.5 times greater than that of Hinosan. BD-15 and BD-17 are also active (activity is greater than Hinosan).

For *H. oryzae* at 48 hours the ED_{50} value increases in

the following order:

BD-16 < BD-15 < BD-13 < BD-11 < BD-18 < BD-10 < BD-17 < BD-14 < Hinosan

i.e., the isopropylamido compound is most effective, but its inhibitory effect is about 1.6 times less than that of Hinosan.

For V. albo-atrum at 48 hours the ED_{50} value increases in the following order:

BD-10 < BD-18 < BD-16 < BD-15 < BD-11 < BD-17 < BD-14 < Hinosan < BD-13

i.e., the dimethylamido compound is the most active, and its inhibitory effect is greater than that of Hinosan. Other compounds are less active.

To sum up, the nonylamido compound is most effective against A. niger; the dimethylamido compound is most active against P. zonsani and V. albo-atrum; and the isopropylamido compound is most effective against H. oryzae.

Table - 11

Fungicidal activity of some amido compounds against A. niger and P. gansene (Spore germination).

Code name of the Compound.	ED_{50} values in μ g/ml			
	Aspergillus niger		Penicillium gensoni	
	24 hours	48 hours	24 hours	48 hours
BD - 10	1096.47	1445.43	912.01	1288.24
BD - 11	1258.92	1412.53	1513.56	1819.70
BD - 13	776.24	1230.26	630.95	891.25
BD - 14	794.32	1174.89	1445.43	1862.08
BD - 15	870.96	1230.26	812.83	1023.29
BD - 16	1548.81	1905.46	1412.53	1949.84
BD - 17	831.76	1000.00	758.57	1023.29
BD - 18	1096.47	1318.25	1096.47	1412.53
Hinosan (Standard)	1047.12	1862.08	891.25	1202.26

[Ref. Tables 11A to 11 I, page - 215 to 223
12A to 12 I, page - 224 to 232.

Table - 12

Fungicidal activity of some amido compounds against *A. niger* and *P. genesne* (Spore germination)

Code name of the Compound	ED_{95} values in μ g/ml			
	Aspergillus niger		Penicillium genesani	
	24 hours	48 hours	24 hours	48 hours
BD - 10	2454.70	3630.78	1862.08	2511.88
BD - 11	1995.26	2630.26	2951.20	3235.93
BD - 13	2290.86	2818.38	1230.26	1548.81
BD - 14	2398.83	3019.95	2884.03	4168.69
BD - 15	1819.70	2754.22	1778.27	2344.22
BD - 16	3162.27	3981.07	3162.27	3801.89
BD - 17	1479.10	1905.46	1584.89	2137.96
BD - 18	2754.22	3388.44	2344.22	2630.26
Hinosan (Standard)	3311.31	5129.61	3187.76	2454.70

(Ref. Tables 12A to 12I, page - 224 to 232)

Table - 13

Fungicidal activity of some amido compounds against H. oryzae and V. albo-atrum (Spore germination).

Code name of the Compound	ED_{50} values in $\mu g/ml$			
	Helminthosporium oryzae		Verticillium albo-atrum	
	24 hours	48 hours	24 hours	48 hours
BD - 10	812.83	1023.29	1737.80	2691.53
BD - 11	1047.12	1258.92	1230.26	1445.43
BD - 13	1071.51	1288.24	630.95	977.23
BD - 14	776.24	831.76	776.24	1318.25
BD - 15	1071.51	1318.25	1230.26	1621.81
BD - 16	1122.01	1412.53	1348.96	1778.27
BD - 17	758.57	977.23	1023.29	1348.96
BD - 18	977.23	1071.51	1698.24	1995.26
Kinosan (Standard)	333.84	588.84	467.73	1122.01

[Ref. Tables - 13A to 13I, page - 233 to 241
14A to 14I, page - 242 to 250]

Table - 14

Fungicidal activity of some amido compounds against H. oryzae and V. albo-atrum (Spore germination).

Code name of the Compound	ED_{95} values in µg/ml			
	Helminthosporium oryzae		Verticillium albo-atrum	
	24 hours	48 hours	24 hours	48 hours
BD - 10	1513.56	1659.68	4570.88	6918.30
BD - 11	1698.24	1905.45	2290.86	2398.83
BD - 13	2137.96	2187.76	1259.92	1819.70
BD - 14	1230.26	1380.38	1584.89	3162.27
BD - 15	2398.83	2630.26	3019.95	3548.13
BD - 16	2454.70	2570.39	3235.93	3388.44
BD - 17	1258.92	2041.73	2290.86	2570.39
BD - 18	1698.24	1778.27	3715.35	4265.79
Hinosan (Standard)	933.25	1148.15	1174.89	3715.35

(Ref. Tables 14A to 14I, page - 233 to 241 / 242 to 250)

11. DISCUSSION ON PROTECTANT ACTIVITY AGAINST H. ORYZAE ON DETACHED RICE LEAVES.

The protectant activity on detached rice leaves against H. oryzae have been carried out by using only two compounds (BD-15 and BD-17). The percent inhibition data have been presented in Table-15.

Table - 15

Code No.	Concentration (μg/ml)	No. of lesions observed after 48 hours.	Percent inhibition of infection.
BD - 15	1500	22	40.54
	1000	26	29.73
	500	31	16.22
BD - 17	1500	14	62.16
	1000	19	48.65
	500	32	13.51
Control	--	37	--

The results reveal that the nonylamido compound (BD-17) at 1500 μg/ml and 1000 μg/ml inhibited the infection by H. oryzae on detached leaves by 62 and 48%, whereas the pyrrolidino compound (BD-15) at the same concentration inhibited the infections by 40 and 29% respectively. The activity in vivo of both the compounds against this pathogen was much lower than that in vitro.

12. DISCUSSION ON PROTECTANT ACTIVITY AGAINST H. ORYZAE ON RICE PLANTS.

The protectant activity on rice plants (40 days old) against H. oryzae have been performed by using pyrrolidino (BD-15) and nonylamido (BD-17) compounds. The percent inhibition of infection data have been given in Table-16.

The nonylamido compound (BD-17) at 1500 μg/ml and 1000 μg/ml inhibited the infection by 56% and 44% respectively, whereas the pyrrolidino compound at the same concentrations inhibited the infection by 51 and 47% respectively.

Table - 16

Protectant activity of BD-15 and BD-17 against Helminthosporium oryzae infection on rice plant (40 days old).

Treatment	Concentration (μg/ml)	Mean no. of spots/ plant(a)	Mean disease index*/ plant(a)	't' at 5% level (P=0.05)	Percent inhibition of infection(b)
BD - 15	1500	6.4	2.212	5.87	51.64
	1000	6.0	2.425	5.24	47.04
	500	9.95	3.80	2.13	16.93
BD- 17	1500	4.8	2.0	8.29	56.28
	1000	5.35	2.525	5.39	44.80
	500	7.45	3.10	3.38	32.24
Control	--	12.30	4.575	--	--

(a) = Average of 20 plants.
(b) = Percentage of inhibition of infection = $\frac{P_n - P_t}{P_n} \times 100$

P_n = Disease index of control plants.

P_t = Disease index of treated plants.

* = The disease index has been calculated by using the method of Chattopadhyay and Bose [Plant Disease Reporter 63, 103 (1979)]

13. DISCUSSION ON PHYTOTOXIC PROPERTIES

13.A. Seed treatment by soaking.

The toxic effects of the compounds (BB-16 and BB-17) on the germination and root and shoot growth of rice (Bharial variety) are presented in Table - 17.

Treatments of seeds with these two compounds have no effect on germination compared to control. However, root and shoot growth have been drastically reduced for both the compounds. The percent decrease of root length over control is greater than that of shoot length.

13. B. Effect of Spraying on Rice Plants.

The toxic effects of these two compounds on the shoot growth of rice plants are given in Table - 18. The shoot growth has been reduced drastically for both the compounds. The highest decrease in shoot growth is observed in case of nonylamido compound; the decrease is 63.7% over control. In general an increase in the concentration decreases shoot growth of the rice plants.

Table - 17

Effect of treatment with BD-15 and BD-17 on germination and root and shoot growth of rice seeds.

(By Seed Soaking)

Code No. of the Compound.	Concentration of the Compound (μg/ml)	Seed soaked for 2 hours				
		% of germination	Mean root length(mm)	% decrease over control	Mean shoot length (mm)	% decrease over control.
BD - 15	1500	92 (96)	7.8 (11.80)	53.01 (51.84)	7.8 (8.50)	37.60 (51.51)
	1000	93 (95)	10.9 (13.22)	34.30 (46.04)	9.5 (9.44)	24.00 (46.15)
	500	92 (96)	12.5 (13.25)	24.70 (45.92)	10.2 (11.22)	18.40 (35.82)
BD - 17	1500	90 (96)	7.42 (9.26)	55.30 (62.20)	6.5 (7.46)	48.00 (57.44)
	1000	92 (96)	8.75 (13.22)	47.29 (53.96)	8.5 (11.59)	32.00 (33.88)
	500	94 (96)	10.25 (13.54)	38.25 (44.73)	9.48 (11.58)	24.16 (33.94)
Control	--	92 (96)	16.60 (24.50)	- (-)	12.50 (17.53)	- (-)

* Results in parenthesis denote the value of 4 hours.

Table - 18

Phytotoxic activity of BD-15 and BD-17 on rice plant (Oryzae sativa).

[By Spraying]

Code name of the Compound	Concentration of the compound (μg/ml)	Total shoot length (cm)[b]		Length increase (cm)	Mean length increase	% decrease over control.	't' value at 5% level (P = 0.05)
		Before Spray	After Spray				
BD - 15	1500	564.1	582.1	18.0	0.36	55.00	6.56
	1000	551.0	571.8	20.8	0.41	48.75	5.57
	500	570.4	594.4	24.0	0.48	40.00	4.26
BD - 17	1500	508.0	522.9	14.9	0.29	63.75	7.96
	1000	537.6	556.8	19.2	0.38	52.50	6.17
	500	543.1	568.0	24.9	0.49	38.75	4.00
Control	--	533.1	573.3	40.2	0.80	--	--

b = a total of fifty plants.

14. GENERAL CONCLUSIONS AND REMARKS:

(i) Some 2-alkylamido-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-sulphides have been prepared by the reaction of the corresponding phosphoramidothioic dichlorides with 5-nitro-saligenin. All compounds are white crystalline solids. Attempts had also been made several times to prepare the 2-methylamido-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-sulphide, and its ethylamido analog; in each case a viscous yellowish liquid was obtained; the pure amidophosphorthionates could not be isolated from these liquid mixtures.

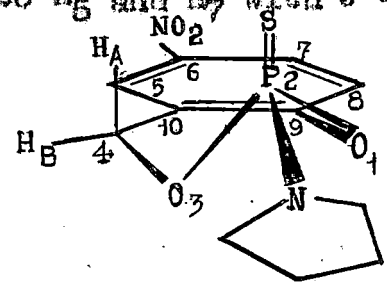
(ii) The structure of the compounds have been determined by chemical analyses and IR, mass and PMR spectra. The spectral data of only four compounds are presented.

The common IR bands for the compounds are : 1010-1030 cm^{-1}(s), P-O-C (alkyl); 1235-1250 cm^{-1}(vs), P-O-C (aryl); 880-920 cm^{-1}(s), P-O-C (aryl); 1515-1520 cm^{-1}(s), asym. str. of nitro group; 1340-1345 cm^{-1}(s), sym. str. of nitrogroup; 800-820 cm^{-1}, P = S (I); 640-660 cm^{-1}, P = S (II). Neither of the two P = S bands shows any systematic shifts which reflect changes in the inductive properties of the substituent; this is not unexpected if they do indeed arise from mixed modes.

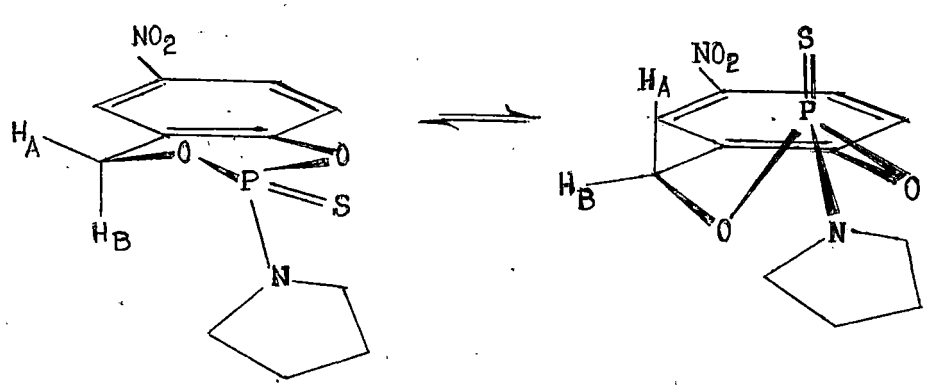
The compounds show parent molecular ion (M^+) peaks in the mass spectra. Fragmentation by loss of 'SH' radical is important; all compounds show an ion due to $(M - SH)^+$, and it is the base peak for all of the four alkylamidophosphorothionates.

Finally the structure of the compounds has been settled by taking PMR spectra; for all compounds the endo-cyclic $-CH_2-$ group gives eight lines in PMR spectra.

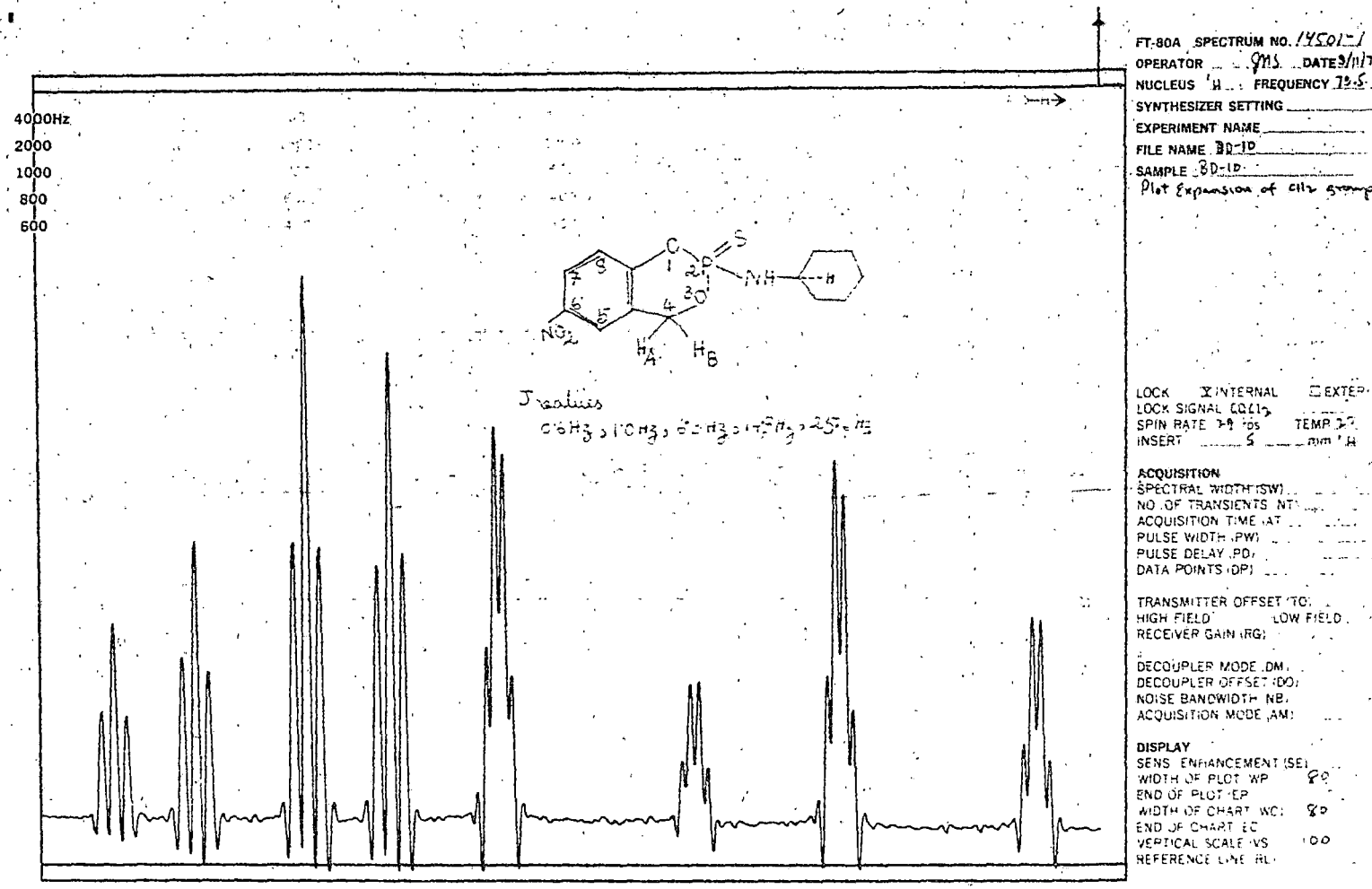
Recently several plot expansions* and decoupling experiments have been performed in our laboratory (Das et al, unpublished results). These experiments, in case of BD-10, suggest that the quasi-equatorial proton H_{4B} is assigned to the 5.2 ppm proton ($J = 25.6$ Hz) and the quasi-axial proton H_{4A} is assigned to the 5.6 ppm. proton ($J = 6.3$ Hz); the geminal coupling constant is 14.7 Hz. It has also been observed that the proton H_{4B} is coupled equally to the three aromatic protons H_5, H_7 and H_8 with $J = 0.6$ Hz; the proton H_{4A} is more strongly coupled to H_5 and H_7 with $J = 1.0$ Hz, but not to H_8



From the temperature dependent PMR spectral study at 270 MHz in the temperature range $-70^{\circ}C$ to $+60^{\circ}C$ of the methoxy compound (BD-8), it is fairly evident that the methylene protons of the hetero ring are not equivalent to each other, and the dioxaphosphoring ring is conformationally mobile in solution (Das et al,



* Graphs are attached (Fig- II. 12, 13).



Plot Expansion of -C- group in diocaphosphorin ring.

H_A H_B

Fig. 11 Plot expansion of the $-CH_2-$ group (in BD-10), 5.0 - 5.8 ppm region.

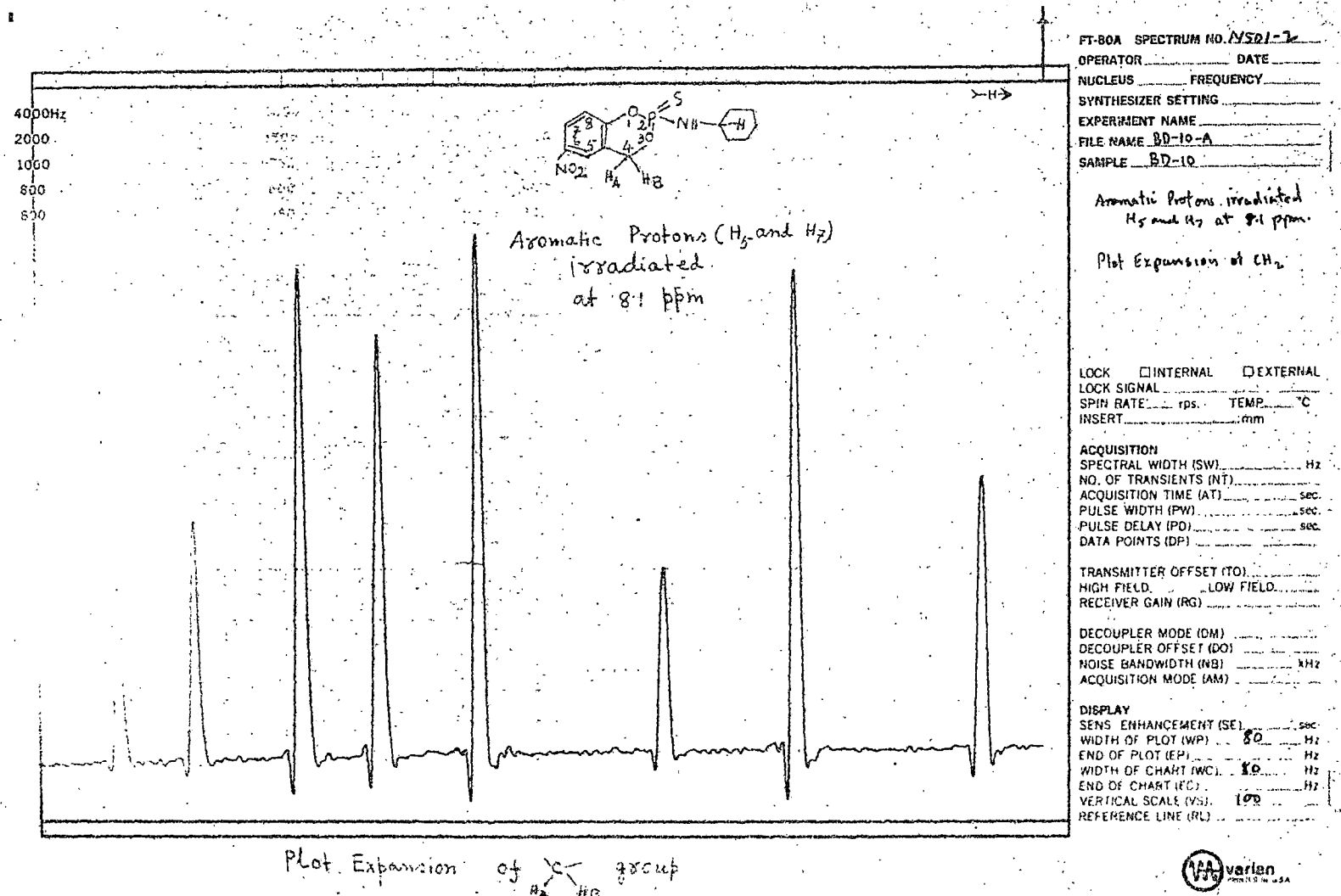
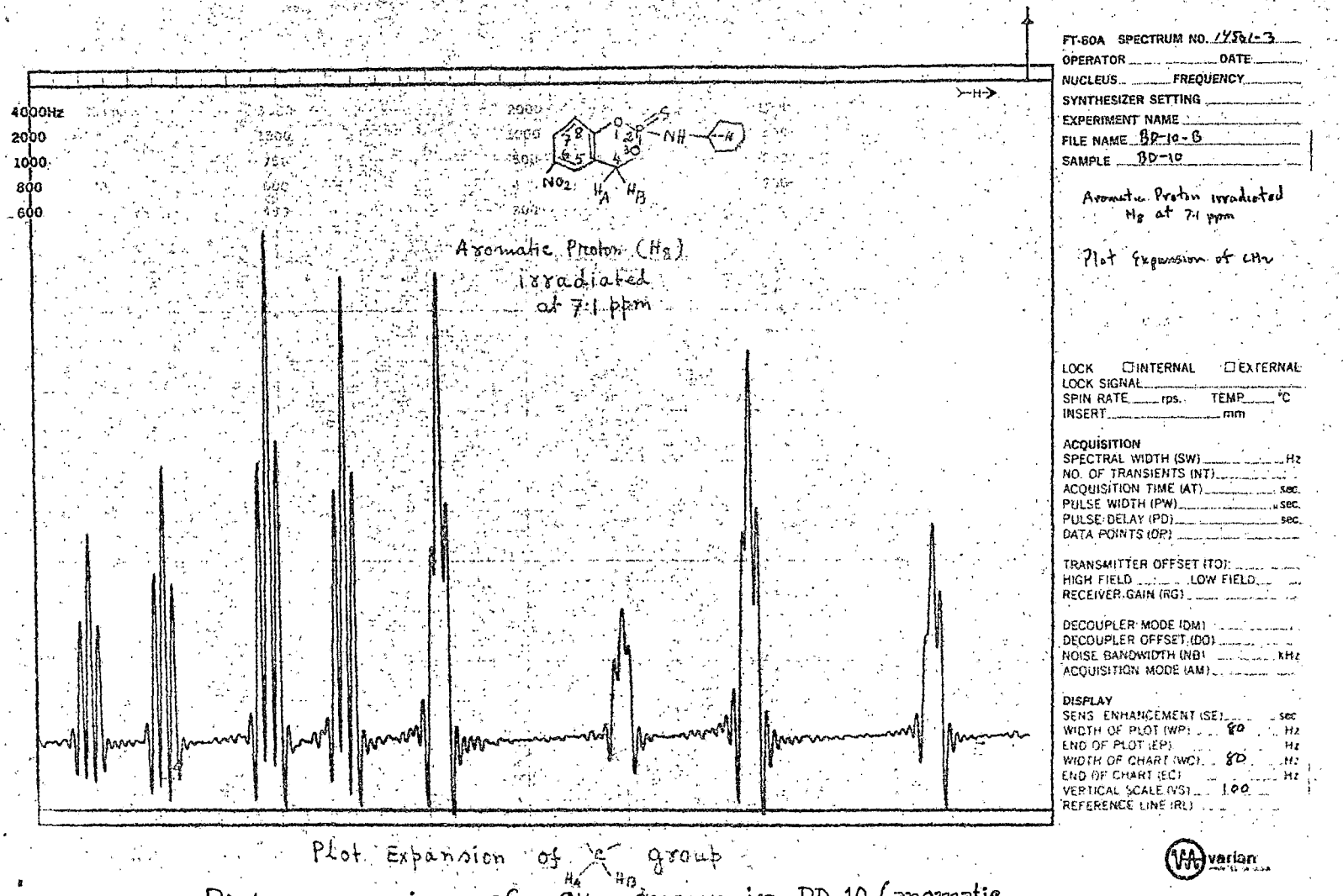


Fig-12. Plot expansion of $-CH_2-$ group in BD-10 (aromatic protons H_5 and H_7 irradiated at 8.1 ppm).



FT-60A SPECTRUM NO. 1956-3
OPERATOR ____ DATE ____
NUCLEUS ____ FREQUENCY ____
SYNTHESIZER SETTING ____
EXPERIMENT NAME ____
FILE NAME BD-10-6
SAMPLE BD-10

Aromatic Proton irradiated
H_8 at 7.1 ppm

Plot Expansion of CH_2

LOCK ☐INTERNAL ☐EXTERNAL
LOCK SIGNAL ____
SPIN RATE ____ rps. TEMP ____ °C
INSERT ____ mm

ACQUISITION
SPECTRAL WIDTH (SW) ____ Hz
NO. OF TRANSIENTS (NT) ____
ACQUISITION TIME (AT) ____ sec.
PULSE WIDTH (PW) ____ μsec.
PULSE DELAY (PD) ____ sec.
DATA POINTS (DP) ____

TRANSMITTER OFFSET (TO) ____
HIGH FIELD ____ LOW FIELD ____
RECEIVER GAIN (RG) ____

DECOUPLER MODE (DM) ____
DECOUPLER OFFSET (DO) ____
NOISE BANDWIDTH (NB) ____ kHz
ACQUISITION MODE (AM) ____

DISPLAY
SENS ENHANCEMENT (SE) ____ sec
WIDTH OF PLOT (WP) 80 Hz
END OF PLOT (EP) ____ Hz
WIDTH OF CHART (WC) 80 Hz
END OF CHART (EC) ____ Hz
VERTICAL SCALE (VS) 100
REFERENCE LINE (RL) ____

varian

Fig-13. Plot expansion of $-CH_2-$ group in BD-10 (aromatic proton H_8 irradiated at 7.1 ppm).

unpublished results).

(iii) The dimethylamido (BD-13) and the morpholino (BD-11) compounds have some insecticidal activity; but their activities are less than that of the 2-methoxy-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-sulphide (BD-8) and Salithion. The other compounds are non-insecticidal.

(iv) All compounds (BD-10 to BD-15) are less toxic to rats than salithion; however, the diethylamido (BD-12), the dimethylamido (BD-13), and the pyrrolidino (BD-15) compounds have greater toxicity compared to other phosphoramidothionates.

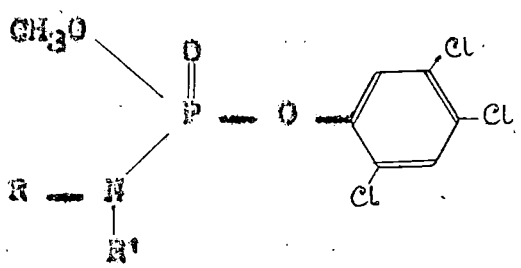
(v) The results of wheat seed germination studies indicate that none of the compounds are phytotoxic upto 500 ppm concentration, however some of the compounds (BD-10, BD-11 and BD-17) show 90 percent germination at 500 ppm.

Treatments of rice seeds with the compounds (BD-15 and BD-17) have no effect on germination; however, root and shoot growth have been drastically reduced. Further studies on the toxic effects of these two compounds (BD-15 and BD-17) on rice plants indicate that the shoot growth has been reduced drastically for both the compounds. Phytotoxic properties of other compounds on rice seeds and plants have not yet been performed.

(vi) It has been observed that for any compounds (BD-10 to BD-14), the housefly-head acetylcholinesterase (HFAChE) is more inhibited than the blood-cholinesterase (blood-ChE). The anticetylcholinesterase

activity of the dimethylamidophosphorothionate (BD-13) is highest, and that of the diethylamidophosphorothionate (BD-12) is least (for HFAChE); but the anticholinesterase activity for blood - ChE of the morpholino compound (BD-11) is most, and that of the isopropylamidophosphorothionate (BD-14) is least.

Hansch and Deutsch (Biochim. Biophys. Acta., 126, 117, 1966) analysed the data obtained by Fukuto et al (J. Econ. Entoml., 56, 808, 1963) from a series of methyl 2,4,5-trichlorophenyl - N - alkyl phosphoramidates in order to clarify the effect of the N - alkyl group on the inhibitory activity of the HFAChE. They observed that



the logarithm of the bimolecular inhibition constant is correlated excellently with Taft's steric constant E_s and polar constant σ^* of the substituent; the bulky isopropyl and tert - butyl groups decrease inhibition rates by steric interference. On the other hand the ring substituents of methyl phenyl N-methyl phosphoramidates directly affect the antiacetylcholinesterase activity (HFAChE) by virtue of the electronic and hydrophobic properties (Neely et al, J. Agric. Food Chem., 16, 571, 1968). However, in a series of ethyl S-(substituted)-phenyl phosphoroamidothiolates, no correlation was observed between the rates of cholinesterase inhibition

bition and any of the free energy parameters for ring substituents (Sanborn, J.R. and Fucuto, T.R; J. Agric. Food. Chem., 20 926, 1972); moreover, the anticholinesterase activity (HFAChE) of phosphoramidothiolates is not always correlated with their insecticidal activity.

In case of nitro-saligenin cyclic phosphoramidothionates, the antiacetylcholinesterase activity (HFAChE) is not correlated with their insecticidal activity. Among the eight compounds (BD-10 to BD-18), only the dimethylamido compound (BD-13) shows highest insecticidal activity, and also highest antiacetylcholinesterase activity. Although antiacetylcholinesterase activity of both dimethylamido and isopropylamido compounds are comparable (I_{50} is 1.80×10^{-5} M and 1.86×10^{-6} M respectively, Table - 6, column - 2, page - 138), the insecticidal activity of the dimethylamido compound is highest ($LC_{100} = 3.6\ \mu g/g$) and that of the isopropylamido compound is least ($LC_{100} = >10 \mu g/g$). When the data for other nitro-saligenin cyclic phosphoramidothionates will be available we will try to find out the correlation between antiacetylcholinesterase activity and E_s, σ^* as well as π values of alkylamido groups.

(vii) From the chemical hydrolysis studies it has been observed that the compounds containing the disubstituted amido groups are extremely resistent to hydrolysis compared to other compounds having the monosubstituted amido groups. It has also been observed that the rate of alkaline hydrolysis is increased as the pH value increases from 7.7 to 11.8.

(viii) From the fungicidal activity studies (by growth inhibition) against P. oryzae, V. albo-atrum, A. solani and H. oryzae indicate that some of the compounds show good inhibitory effect on the growth of different fungi; however, compared to Hinosan they have less inhibitory effect. Among the nine compounds, the dimethylamido compound is most active against P. oryzae, the isopropylamido compound is against H. oryzae, and the cyclohexylamido compound is against V. albo-atrum & A. solani.

From the spore germination inhibition studies against A. niger, P. ramseni, V. albo-atrum and H. oryzae, it has been observed that all compounds are effective. The nonylamido compounds is most active for A. niger, and the dimethylamido compound is against P. ramseni & V. albo-atrum; their activities are greater than that of Hinosan. The isopropylamido compound is most effective against H. oryzae; but, its activity is less than that of Hinosan.

Protectant activity studies (in vivo) against H. oryzae on detached rice leaves and rice plants by using only two compounds (BD-15 and BD-17) indicate that the activity of nonylamido compound (BD-17) is greater than that of the pyrrolidino compound (BD-15). Protectant activity studies for other compounds have not yet been performed.

(ix) The antifungal activity data justify further examination of these phosphoramidothionates as potential fungicides with special reference to the selectivity of their action. Whether the use of these compounds will protect the plants from diseases in the field remains to be studied.

In order to find out the chemical structure — biological activity relationship in these nitro-saligenin cyclic phosphoramidothionates, we have to synthesize several new compounds in which the nitro group is to be incorporated in different position of the aromatic ring, and to investigate their biological activities, phytotoxic properties, fungicidal activities, anti - ChE enzyme activities, and other toxicological properties including delayed neurotoxicity in hens.

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TABLES

Table - 1A

Effect of BD - 10 on the growth of *Pyricularia oryzae*.

Concentration (μg/ml)	Percentage of inhibition over control after			
	48 hours	72 hours	96 hours	120 hours
200	100	88.23*	77.55*	72.60*
150	100	79.41*	69.38*	65.75*
100	100	76.47*	63.38*	58.49*
50	92.50*	60.29*	50.65*	42.75*
25	64.00*	46.45*	36.75*	30.50*
12.5	50.00*	30.75*	24.45*	16.45*
5	25.50*	19.60	11.25	8.75
ED_{95} =	60.26	275.42	537.03	616.59
ED_{50} =	12.30	30.19	47.86	66.06
Regression constants: $Y = mx + c$				
m =	64.840	46.486	43.104	46.145
c =	- 20.780	- 18.836	- 22.745	- 34.074
r =	0.993	0.997	0.999	0.999

* Data used for regression analysis.

Table - 1B

Effect of BD - 11 on the growth of Pyricularia oryzae

Concentration (µg/ml)	Percentage of inhibition over control after			
	48 hours	72 hours	96 hours	120 hours
200	100	100	90.47*	78.61*
150	100	85.29*	82.85*	70.07*
100	100	76.47*	71.42*	61.66*
50	73.91*	58.75*	52.38*	45.92*
25	56.82*	44.17*	38.09*	25.61*
12.5	43.47*	29.41*	23.50*	19.35*
5	24.50*	17.35	12.45	8.70
ED_{95} =	138.03	229.08	251.18	389.04
ED_{50} =	16.59	30.90	38.90	61.65
Regression constants: Y = mx + c				
m =	48.866	51.955	55.717	56.725
c =	- 9.698	- 27.844	- 39.020	- 51.970
r =	0.998	0.999	0.997	0.988

* Data used for regression analysis.

Table - 16

Effect of BD - 12 on the growth of *Pyricularia oryzae*

Concentration (µg/ml)	Percentage of inhibition over control after			
	48 hours	72 hours	96 hours	120 hours
200	100	100	100	100
150	100	100	94.36*	87.60*
100	100	100	84.00*	76.92*
50	86.33*	70.58*	64.90*	58.15*
25	70.91*	56.70*	50.27*	40.50*
12.5	51.50*	36.75*	30.50*	20.75*
6	32.33*	17.64*	9.09	7.65
ED_{95} =	69.18	134.39	151.35	194.98
ED_{50} =	10.47	19.49	25.11	35.48
Regression constants: Y = mx + c				
m =	54.894	54.051	57.622	61.414
c =	- 6.319	- 20.255	- 31.186	- 45.664
r =	0.998	0.997	0.999	0.999

* Data used for regression analysis

Table - 19

Effect of ED - 13 on the growth of Pyricularia oryzae

Concentration (µg/ml)	Percentage of inhibition over control after			
	48 hours	72 hours	96 hours	120 hours
200	100	100	100	100
150	100	100	100	100
100	100	100	100	91.83*
50	100	100	93.80*	70.65*
25	100	93.76*	71.50*	47.50*
12.5	92.50	71.23*	60.00*	26.75*
5	68.25	44.30*	22.50*	21.50*
ED_{95} =	>12.50 <25.00	25.70	48.97	134.89
ED_{50} =	<5.00	5.88	10.96	21.87
Regression constants: Y = mx + c				
m =		70.477	68.958	56.497
c =		- 4.707	- 21.835	- 25.868
r =		0.999	0.988	0.971

*Data used for regression analysis

Table - 11

Effect of BD - 14 on the growth of Pyricularia oryzae.

Concentration (µg/ml)	Percentage of inhibition over control after			
	48 hours	72 hours	96 hours	120 hours
200	100	100	100	100
150	100	100	100	100
100	100	100	100	100
50	100	100	100	88.13
25	100	63.63	52.77	43.47
12.5	100	42.00	32.50	17.39
5	53.60	18.18	16.66	- -
ED_{95} =	>5.00 <12.50	>25.00 <50.00	>25.00 <50.00	>50.00 <100.00
ED_{50}	<5.00	>12.50 <25.00	>12.5 <25.00	>25.00 <50.00

Table - 1F

Effect of BD - 15 on the growth of Pyricularia oryzae

Concentration (µg/ml)	Percentage of inhibition over control after			
	48 hours	72 hours	96 hours	120 hours
200	100	100	100	100
150	100	100	100	92.39*
100	100	100	96.50*	78.90*
50	90.50*	80.01*	76.46*	60.65*
25	71.25*	62.45*	58.35*	42.86*
12.5	51.42*	46.05*	44.60*	25.35*
5	30.25*	24.25*	13.15*	6.30
ED_{95} =	56.88	91.20	93.32	169.82
ED_{50}	10.71	14.12	17.87	31.62
Regression constants: Y = mx + c				
m =	60.543	55.609	61.941	61.161
c =	- 12.705	- 14.375	- 27.892	- 41.999
r =	0.998	0.999	0.996	0.998

*Data used for regression analysis

Table - 18

Effect of BD - 18 on the growth of Pyricularia oryzae

Concentration (μg/ml)	Percentage of inhibition over control after			
	48 hours	72 hours	96 hours	120 hours
200	100	100	86.50*	80.25
150	100	79.75*	78.58*	71.50*
100	100	72.72*	67.72*	60.06*
50	66.66*	54.54*	52.83*	48.00*
25	52.38*	40.45*	34.73*	24.37*
12.5	33.33*	23.30*	15.50*	11.63
6	12.50*	9.75	6.25	4.60
ED_{95} =	154.38	275.42	281.83	363.07
ED_{50} =	23.44	38.01	45.70	61.65
Regression constants: Y = mx + c				
m =	54.979	62.533	57.606	58.654
c =	- 25.682	- 33.473	- 46.193	- 55.193
r =	0.988	0.999	0.999	0.993

* Date used for regression analysis

Table - III

Effect of BD - 17 on the growth of Pyricularia oryzae

Concentration (µg/ml)	Percentage of inhibition over control after			
	48 hours	72 hours	96 hours	120 hours
200	100	100	100	95.23*
150	100	100	100	86.50*
100	100	88.21*	84.75*	75.30*
50	88.23*	73.68*	65.15*	56.15*
25	70.58*	55.28*	47.25*	36.35*
12.5	50.50*	45.80*	34.50*	14.25*
5	29.41*	15.05*	11.25*	8.35
ED_{95} =	63.09	123.02	158.48	194.98
ED_{50} =	11.22	18.19	25.11	40.73
Regression constants: Y = mx + c				
m =	59.375	54.166	65.689	66.113
c =	-12.461	-18.512	-27.989	-56.611
r =	0.998	0.998	0.996	0.999

* Data used for regression analysis

Table - I I

Effect of ED-18 on the growth of Pyricularia oryzae

Concentration (µg/ml)	Percentage of inhibition over control after			
	48 hours	72 hours	96 hours	120 hours
200	100	100	100	100
150	100	100	95.45*	90.90*
100	100	92.00*	83.30*	77.75*
50	96.42*	75.00*	65.75*	56.23*
25	78.57*	57.50*	46.63*	36.69*
12.5	57.90*	40.00*	27.54*	16.25*
5	32.00*	16.50*	10.20	6.35
ED_{95} =	45.70	138.03	147.91	177.82
ED_{50} =	9.12	11.22	27.54	38.01
Regression constants: Y = mx + c				
m =	64.861	38.124	62.135	68.00
c =	- 12.584	- 9.892	- 39.991	- 58.040
r	0.999	0.913	0.999	0.999

* Data used for regression analysis

Table - II

Effect of Hinosan on the growth of Pyricularia oryzae

Concentration (µg/ml)	Percentage of inhibition over control after			
	48 hours	72 hours	96 hours	120 hours
200	100	100	100	100
150	100	100	100	100
100	100	100	100	100
50	100	100	100	100
25	100	100	91.50	87.50
12.5	100	91.73	82.85	78.75
5	84.61	76.85	68.57	62.50
ED_{95} =	>15.00	>12.50	>25.00	>25.00
	<18.50	<25.00	<50.00	<50.00
ED_{50} =	<5.00	<5.00	<5.00	<5.00

Table - 6A

Acetylcholinesterase Inhibition in House-fly head homogenate (HFAChE) at 30°C of BD - 10

(Phosphate buffer, pH = 8.0; total volume = 5.15 ml/6 fly head, λ = 625 nm; in-cubation time 30 min.).

Sets	Inhibition* Conc.(μg)	O. D	$\triangle$ O.D.	% Inhibition (Y)	I_{50} (M)
Control	-	0.67	0	-	
1	60	0.26	0.32	66.13	
2	50	0.28	0.29	50.87	
3	40	0.32	0.28	43.85	2.8611×10^{-5}
4	30	0.36	0.22	38.60	
5	20	0.405	0.165	28.90	

X* = log (Conc. of the inhibitor in μg).

Regression constants : Y = mx + c

c = -44.5645

m = 56.1474

r = 0.9963

Table - 6B

Acetylcholinesterase Inhibition in House-fly head homogenate (HFAChE) at 30°C of BD-11

(Phosphate buffer; pH = 8.0; total volume = 5.15 ml/5 fly head, λ = 625 nm; in-cubation time 30 min).

Sets	Inhibition* Conc.(μg)	O. D.	Δ O.D.	% Inhibition (Y)	I_{50} (M)
Control	(-)	0.610	0	-	
1	50	0.310	0.300	39.21	
2	40	0.340	0.170	33.35	
3	30	0.370	0.140	27.45	5.6326×10^{-5}
4	20	0.415	0.095	18.60	
5	10	0.480	0.030	5.88	

X* = log (Conc. of the inhibitor in μg).

Regression constants : $Y = mx + c$

$c = -42.0753$

$m = 47.3029$

$r = 0.9933$

Table - 6C

Acetylcholinesterase Inhibition in House-fly head homogenate (HFAChE) at 30°C of ES - 12.

(Phosphate buffer; pH = 8.0; total volume 5.15 ml/5 fly head, λ = 625 nm; in-cubation time 30 min).

Sets	Inhibitor* Conc. (μg)	O.D.	ΔO.D.	%Inhibition (Y)	I_{50} (M)
Control	(-)	1.05	0	-	
1	100	0.57	0.48	46.71	
2	80	0.59	0.46	43.81	
3	60	0.64	0.41	39.09	8.2601×10^{-5}
4	40	0.71	0.34	32.38	
5	20	0.80	0.25	23.81	
6	10	0.92	0.13	12.38	

X* = log (Conc. of the inhibitor in μg).

Regression constants ; $Y = mx + c$

$c = -20.6648$

$m = 33.5095$

$r = 0.9985$

Table - 6D

Acetylcholinesterase Inhibition in House-fly head homogenate (HFAChE) at 30°C of BD-13.

(Phosphate buffer; pH = 8.0; total volume 5.15 ml/5 fly head, λ = 625 nm; in-cubation time 30 min).

Sets	Inhibition* Conc.(μg)	O.D.	ΔO.D.	%Inhibition (Y)	I_{50}(M)
Control	(-)	1.05	0	-	
1	100	0.21	0.84	80.00	
2	80	0.27	0.78	74.28	
3	60	0.33	0.72	68.57	1.80×10^{-5}
4	40	0.42	0.63	60.00	
5	20	0.58	0.47	44.76	

X* = log (Conc. of the inhibitor in μg).

Regression constants : $Y = mx + c$

$c = -20.0339$

$m = 49.8322$

$r = 0.9997$

Table - 6B

Acetylcholinesterase Inhibition in House-fly head homogenate (HFAChE) at 30°C of BL-14.

(Phosphate buffer, pH = 8.0; total volume 5.15 ml/5 fly head; λ = 625 nm; in-cubation time 30 min.)

Sets	Inhibitor Conc. (μg)	O.D.	ΔO.D.	%Inhibition (Y)	I_{50} (M)
Control	(-)	1.05	0	-	
1	50	0.41	0.64	60.95	
2	40	0.46	0.59	56.13	1.8642×10^{-5}
3	30	0.61	0.54	51.42	
4	20	0.59	0.46	43.81	
5	10	0.71	0.36	32.38	

X* = log (Conc. of the inhibitor in μg).

Regression constants : Y = mx + c

c = -3.5653

m = 40.6143

r = 0.9993

Table - 6F

Acetylcholinesterase Inhibition in goat-whole blood of BD-10 at 30°C

(Phosphate buffer, pH = 8.0; total volume = 5.15 ml/0.2 ml blood; λ = 625 nm; in cubation time 30 min.).

Sets	Inhibitor* Conc. (μg)	O.D.	$\triangle$O.D.	% Inhibition (Y)	I_{50} (M)
Control	(-)	0.69	-		
1	30	0.63	0.06	8.69	
2	40	0.60	0.09	13.04	
3	60	0.56	0.13	18.84	1.310×10^{-4}
4	80	0.516	0.175	25.56	
5	100	0.48	0.21	30.43	
6	120	0.46	0.23	33.33	

X* = log (Conc. of the inhibitor in μg).

Regression constants : $Y = mx + c$

$c = -53.3108$

$m = 41.7679$

$r = 0.9759$

Table - 69

Acetylcholinesterase Inhibition in goat whole blood of BD-11 at 30°C.

(Phosphate buffer; pH = 8.0; total volume = 5.15 ml/0.2 ml blood; λ = 625 nm; in cubation time 30 min.)

Sets	Inhibitor* Conc. (μg)	O. D.	ΔO.D.	% Inhibition (Y)	I_{50} (M)
Control	(-)	0.80	-		
1	30	0.69	0.11	13.75	
2	40	0.65	0.15	18.75	
3	60	0.58	0.22	27.50	1.0426×10^{-4}
4	80	0.54	0.26	32.50	
5	100	0.49	0.31	38.75	
6	120	0.46	0.35	43.75	

X* = log (Conc. of the inhibitor in μg).

Regression constants : Y = mx + c

c = -59.8146

m = 49.2530

r = 0.9968

Table - 6H

Acetylcholinesterase Inhibition in goat whole blood of BD-12 at 30°C.

(Phosphate buffer, pH = 8.0; total volume = 5.15 ml/0.2 ml blood; λ = 625 nm; in-cubation time 30° min.).

Sets	Inhibitor* Conc. (μg)	O. D.	ΔO.D.	% Inhibition (Y)	I_{50} (M)
Control	(-)	0.65	(-)		
1	40	0.59	0.06	9.23	
2	60	0.53	0.12	18.46	1.4259×10^{-4}
3	80	0.48	0.17	26.15	
4	100	0.45	0.20	30.76	
5	120	0.42	0.23	35.38	

X* = log (Conc. of the inhibitor in μg).

Regression constants : Y = mx + c

c = -73.8637

m = 54.9211

r = 0.9995

Table - 6I

Acetylcholinesterase Inhibition in goat-whole blood of BD-13 at 30°C.

(Phosphate buffer, pH = 8.0; total volume = 5.15 ml/0.2 ml blood, λ = 625 nm; in-cubation time 30 min.).

Sets	Inhibition* Conc. (μg)	O. D.	ΔO.D.	% Inhibition (Y)	I_{50} (M)
Control	(-)	0.69	-		
1	30	0.66	0.04	6.15	
2	40	0.62	0.07	10.76	
3	60	0.58	0.11	15.94	4.6564×10^{-4}
4	80	0.55	0.14	20.28	
5	100	0.53	0.16	23.18	
6	120	0.51	0.18	26.08	

X* = log (Conc. of the inhibitor in μg).

Regression constants : $Y = mx + c$

$c = -41.7889$

$m = 32.5769$

$r = 0.9996$

Table - 6J

Acetylcholinesterase Inhibition in goat-whole blood of ED-14 at 30°C

(Phosphate buffer, pH = 8.0; total volume = 5.15 ml/0.2 ml blood; λ = 625 nm; incubation time 30 min.).

Sets	Inhibitor* Conc.(μg)	O.D.	ΔO.D.	% Inhibition (Y)	I_{50} (M)
Control	(-)	0.55	-		
1	50	0.47	0.08	14.54	
2	100	0.42	0.13	23.63	
3	150	0.39	0.16	29.09	4.7805×10^{-4}
4	200	0.37	0.18	32.72	
5	250	0.33	0.20	36.36	

X* = log (Conc. of the inhibitor in μg).

Regression constants : $Y = mx + c$

$c = -33.0805$

$m = 30.8911$

$r = 0.9996$

Table - 7A

Chemical hydrolysis of BD-11 at λ =410 nm; pH = 11.85; 0.0095 M NaOH (in 50% Ethanol) ϵ = 20090, Initial concentration, C_o = 7.06 x 10^{-5} M. temperature 20°C.

Time (hrs) (X)	O. D.	$C_t \times 10^5$ (M)	$\log \frac{C_o}{C_o - C_t}$ (Y)	K_{hyd} (min^{-1})
5	0.103	0.51269	0.0327	
27.75	0.316	1.5729	0.1095	
52.50	0.445	2.2150	0.1636	6.1413×10^{-5}
75.33	0.49	2.4390	0.1841	
124.6	0.6	2.9866	0.2388	

Regression constants : $Y = mx + c$

$c = 0.0526$

$m = 0.0016$

$r = 0.9599$

Table - 73

Chemical hydrolysis of BD - 13 at λ=410 nm, pH = 11.85, 0.0095 M NaOH (in 50% Ethanol) ϵ = 20000, Initial Concentration, $C_o = 7.3 \times 10^{-5}$; temperature 20°C.

Time (hrs) (X)	O. D.	$C_t \times 10^5$ (M)	$\log \frac{C_o}{C_o - C_t}$ (Y)	K_{hyd} (min^{-1})
6	0.034	0.16924	0.0102	
28	0.143	0.71180	0.0446	
52.75	0.210	1.0463	0.0671	2.687×10^{-5}
76.25	0.255	1.2693	0.0830	
124.75	0.305	1.5182	0.1013	

Regression constants : $Y = mx + c$

$c = 0.0193$

$m = 0.0007$

$r = 0.9559$

Table - 7C

Chemical hydrolysis of BD - 15 at λ = 410 nm, pH = 11.85, 0.0095 M NaOH (in 50% Ethanol), Initial concentration, $C_o = 2.12 \times 10^{-5}$ M, temperature 20°C.

Time (hrs) (X)	O. D.	$C_t \times 10^5$	$\log \frac{C_o}{C_o - C_t}$ (Y)	K_{hyd} (min^{-1})
150	0.063	0.31359	0.0695	1.7784×10^{-5}

* This compound (BD-15) was extremely resistant to hydrolysis at pH 11.85; only after 150 hours reading was taken at λ =410 nm and K_{hyd} was calculated from the reading with the help of 1st order rate equation.

$$K = \frac{1}{t} \mu \frac{C_o}{C_o - C_t} \quad .$$

Table - 7D

Chemical hydrolysis of BD-16 at λ = 410, pH = 11.85, 0.0095 M NaOH (in 50% Ethanol), Initial concentration, C_o = 1.67 x 10^{-5} M, temperature 20°C.

Time (hrs) (X)	O. D.	C_t x 10^5 (M)	$\log \frac{C_o}{C_o - C_t}$ (Y)	K_{hyd} (min^{-1})
150	0.044	0.21201	0.0474	1.2129 x 10^{-5}

* This compound (BD-16) also is extremely resistant to hydrolysis at pH 11.85; only after 150 hours reading was taken at λ = 410 nm and K_{hyd} was calculated from the reading with the help of 1st order rate equation:

$$K = \frac{1}{t} \ln \frac{C_o}{C_o - C_t}.$$

Table - 75

Chemical hydrolysis of RR-17 at λ = 410 nm, pH = 11.85, 0.0096 M NaOH (in 50 50% Ethanol) ϵ = 20030, Initial concentration, C_o = 3.14 x 10^{-5} M, temperature 20°C.

Time (min) (X)	O.D.	$C_t \times 10^5$ (M)	$\log \frac{C_o}{C_o - C_t}$ (Y)	K_{hyd} (min^{-1})
5.25	0.07	0.34843	0.0511	
6	0.077	0.38323	0.0565	
10	0.127	0.63216	0.0976	
15	0.177	0.88104	0.1430	20.266 x 10^{-3}
20	0.227	1.085	0.1852	
25	0.265	1.3191	0.2362	
30	0.290	1.4783	0.2764	
35	0.333	1.6575	0.3259	
40	0.358	1.7670	0.3593	
45	0.378	1.8815	0.3971	

Regression constants: $Y = mx + c$

$c = 0.0083$

$m = 0.0088$

$r = 0.9911$

Table - 8A

Effect of ED - 10 on growth of Verticillium-albo - atrum

Concentration (µg/ml)	Percentage of inhibition over control after		
	24 hours	48 hours	62 hours
500	100	93.05	91.37*
400	100	88.54*	83.05*
300	90.62*	75.35*	70.45*
250	85.41*	69.50*	66.50*
200	81.25*	62.35*	58.75*
150	66.50*	51.50*	48.65*
100	52.08*	39.60*	34.40*
50	27.00*	19.82	12.30
ED_{95} =	316.22	501.18	549.54
ED_{50} =	91.20	138.03	154.88
Regression constants:	Y = mx + c		
m =	83.755	79.718	81.187
c =	- 114.606	- 120.719	- 128.023
r =	0.997	0.998	0.998

* Data used for regression analysis

Table - 88

Effect of BD - 11 on growth of Verticillium albo-atrum.

Concentration (µg/ml)	Percentage of inhibition over control after		
	24 hours	48 hours	62 hours
500	100	100	88.27*
400	100	90.35*	79.50*
300	100	77.75*	66.35*
250	100	69.25*	58.75*
200	90.75*	61.35*	48.90*
150	80.00*	49.90*	41.50*
100	62.25*	37.50*	24.75*
50	34.00*	21.65	9.75
ED_{95}	213.79	457.08	588.84
ED_{50}	70.79	141.25	190.54
Regression constants:	Y = mx + c		
m =	93.403	88.507	91.892
c =	- 123.053	- 141.133	- 158.464
r =	0.998	0.997	0.997

* Data used for regression analysis.

Table - 8C

Effect of BD - 12 on growth of *Verticillium albo-atrum*.

Concentration (µg/ml)	Percentage of inhibition over control after		
	24 hours	48 hours	62 hours
500	100	87.90*	78.70*
400	93.75*	78.60*	70.83*
300	82.80*	67.15*	60.30*
250	70.31*	57.30*	50.16*
200	63.50*	51.10*	44.50*
150	50.25*	40.70*	30.16*
100	32.81*	23.50*	21.06
50	24.90	17.65	6.66
ED_{95} =	393.10	588.84	707.94
ED_{50} =	144.54	180.54	234.42
Regression constants:	Y = mx + c		
m =	102.254	91.872	92.702
c =	- 171.830	- 160.096	- 170.110
r =	0.998	0.998	0.997

* Data used for regression analysis.

Table - 8D

Effect of BD - 13 on growth of *Verticillium albo-atrum*.

Concentration (μg/ml)	Percentage of inhibition over control after		
	24 hours	48 hours	62 hours
500	100	100	87.98*
400	100	87.65*	80.01*
300	92.03*	76.50*	68.40*
250	82.30*	68.40*	59.90*
200	75.22*	60.75*	53.50*
150	64.50*	48.35*	40.50*
100	48.24*	32.25*	26.50*
50	19.50*	11.25	7.35
ED_{95} =	323.59	467.73	575.43
ED_{50} =	104.71	151.35	181.97
Regression constants:	Y = mx + c		
m =	91.492	92.582	89.695
c =	- 134.907	- 152.629	- 153.421
r =	0.999	0.999	0.999

* Data used for regression analysis.

Table - 8E

Effect of BD - 14 on growth of Verticillium albo-atrum

Concentration (µg/ml)	Percentage of inhibition over control after		
	24 hours	48 hours	62 hours
500	100	93.00*	86.48*
400	100	87.77*	78.68*
300	88.33*	75.25*	65.72*
250	80.00*	66.66*	56.79*
200	73.33*	56.25*	48.75*
150	62.00*	46.50*	36.25*
100	48.66*	34.75*	20.15*
50	20.15*	15.50	9.25
ED_{95} =	354.81	501.18	538.84
ED_{50} =	104.71	154.88	199.52
Regression constants :	Y = mx + c		
m =	86.077	88.487	95.732
c =	- 124.709	- 144.355	- 173.409
r =	0.999	0.996	0.999

* Data used for regression analysis.

Table - 8F

Effect of BD - 15 on growth of Verticillium albo-atrum

Concentration (µg/ml)	Percentage of inhibition over control after		
	24 hours	48 hours	62 hours
500	100	90.30*	87.50*
400	100	81.81*	78.04*
300	88.31*	72.72*	69.35*
250	80.39*	64.35*	60.45*
200	73.15*	55.55*	53.50*
150	62.00*	43.25*	39.90*
100	49.75*	28.50*	27.65*
50	30.25*	21.65	13.25
ED_{95} =	380.13	537.03	602.55
ED_{50} =	95.49	169.82	181.97
Regression constants : Y = mx + c			
m =	74.073	90.154	87.379
c =	- 86.764	- 151.693	- 147.079
r =	0.996	0.999	0.998

* Data used for regression analysis.

Table - 8C

Effect of ED - 16 on growth of Verticillium albo-atrum.

Concentration (µg/ml)	Percentage of inhibition over control after		
	24 hours	48 hours	62 hours
500	100	95.06*	89.02*
400	100	88.47*	80.62*
300	100	75.45*	68.65*
250	87.14*	68.60*	60.25*
200	80.00*	58.25*	52.50*
150	66.50*	46.00*	38.15*
100	49.75*	30.75*	22.35*
50	20.00*	16.20	6.25
ED_{95} =	238.40	467.73	594.54
ED_{50} =	97.72	158.48	190.54
Regression constants :	Y = mx + c		
m =	96.923	95.133	97.189
c =	- 143.830	- 159.792	- 171.963
r =	0.999	0.999	0.999

* Data used for regression analysis.

Table - 8H

Effect of BD - 17 on growth of Verticillium albo-atrum.

Concentration (µg/ml)	Percentage of inhibition over control after		
	24 hours	48 hours	62 hours
500	100	88.83*	83.25*
400	88.11*	79.61*	73.43*
300	78.25*	69.25*	60.25*
250	70.50*	62.75*	50.90*
200	63.25*	54.60*	42.30*
150	52.40*	44.25*	27.84*
100	37.25*	29.12*	16.25
50	27.15	16.50	8.00
ED_{95} =	467.73	588.84	630.95
ED_{50} =	138.03	173.78	234.42
Regression constants : Y = mx + c			
m =	85.070	85.307	106.079
c =	- 132.550	- 141.343	- 202.152
r =	0.999	0.999	0.999

* Data used for regression analysis.

Table - 8I

Effect of BD - 18 on growth of Verticillium albo-atrum.

Concentration (g/ml)	Percentage of inhibition over control after			
	24 hours	48 hours	62 hours	96 hours
500	83.88*	80.45*	75.60*	
400	77.77*	72.93*	68.20*	
300	69.44*	63.42*	57.25*	
250	63.74*	56.25*	53.75*	
200	56.25*	50.20*	44.75*	
150	46.25*	40.25*	34.25*	
100	34.25*	27.25*	20.50*	
50	21.15	13.20	7.25	
ED_{95} =	676.08	758.57	851.13	
ED_{50} =	162.12	194.98	229.08	
Regression constants: Y = mx + c				
m =	72.653	76.758	79.754	
c =	- 110.846	- 126.435	-138.780	
r =	0.999	0.999	0.998	

* Data used for regression analysis.

Table - 8J

Effect of Hinosan on growth of Lactobacillus elbo-atrum.

Concentration (μ g/ml)	Percentage of inhibition over control after			
	24 hours	48 hours	62 hours	96 hours
500	100	100	100	
400	100	100	100	
300	100	94.66*	86.50*	
250	100	89.04*	80.50*	
200	90.45	78.15*	70.25*	
150	71.65	66.25*	57.28*	
100	63.25	50.65*	40.45*	
50	41.30	22.60*	13.50*	
ED_{95} =	>200.00	295.12	346.73	
ED_{50} =	>50.00	95.49	123.02	
Regression constants : Y = mx + c				
m =		93.313	99.608	
c =		-135.596	-158.216	
r =		0.999	0.999	

* Data used for regression analysis.

Table - 9A

Effect of BD.10 on growth of Alternaria solani

Concentration (μg/ml)	Percentage of inhibition over control after			
	24 hours	48 hours	72 hours	96 hours
500	100	100	100	90.50*
400	100	100	90.50*	83.05*
300	100	100	81.75*	72.25*
250	100	100	74.25*	65.25*
200	100	82.05*	66.92*	55.35*
150	82.15	71.79*	56.51*	46.41*
100	71.85	58.97*	44.05*	42.52*
50	41.50	35.00*	23.60	14.79
ED_{95} =	> 150.00	288.40	446.68	588.84
ED_{50} =	> 50.00	75.35	120.22	147.91
Regression constants : Y = mx + c				
m =		76.974	78.755	74.133
c =		- 95.075	- 113.847	- 110.968
r =		0.999	0.999	0.983

* Data used for regression analysis.

Table - 23

Effect of ED - 11 on growth of *Alternaria solani*

Concentration (µg/ml)	Percentage of inhibition over control after			
	24 hours	48 hours	72 hours	96 hours
500	100	100	85.29*	80.43*
400	100	90.42*	77.58*	70.20*
300	100	82.14*	64.50*	57.60*
250	100	72.61*	54.94*	48.91*
200	100	63.50*	49.00*	40.82*
150	71.07	57.90*	36.17*	27.25*
100	53.16	50.00*	20.25*	16.15
50	36.85	37.50*	11.35	8.15
ED_{95} =	>150.00	675.43	616.59	676.08
ED_{50} =	<100.00	147.91	208.92	245.47
Regression constants : Y = mx + c				
m =		76.239	95.417	101.306
c =		- 116.148	- 171.444	- 192.649
r =		0.996	0.999	0.999

* Data used for regression analysis.

Table - 35

Effect of ED - 12 on growth of Alternaria solani

Concentration (μ g/ml)	Percentage of inhibition over control after			
	24 hours	48 hours	72 hours	96 hours
500	100	100	100	90.50*
400	100	100	90.50*	80.75*
300	100	85.20*	78.75*	68.18*
250	100	77.75*	71.42*	60.36*
200	77.77*	70.37*	62.63*	50.00*
150	66.50*	56.50*	50.25*	36.36*
100	52.55*	40.03*	34.86*	22.50*
50	25.25*	18.36	12.30	7.75
ED_{95} =	309.02	363.07	436.51	549.54
ED_{50} =	93.32	125.89	144.54	194.98
Regression constants: Y = mx + c				
m =	85.933	98.600	93.296	99.919
c =	- 119.786	- 152.925	- 151.868	- 178.859
r =	0.999	0.999	0.999	0.999

* Data used for regression analysis.

Table - 9D

Effect of BD - 13 on growth of Alternaria solani

Concentration (g/ml)	Percentage of inhibition over control after			
	24 hours	48 hours	72 hours	96 hours
500	100	100	78.50*	69.50*
400	100	100	70.50*	61.50*
300	100	79.72*	59.65*	50.65*
250	100	72.72*	52.00*	44.55*
200	100	64.50*	44.25*	36.75*
150	100	52.50*	32.45*	26.15*
100	68.50	37.65*	18.50*	12.08
50	37.15	19.15	10.20	6.25
ED_{95} =	>100.00	426.57	741.31	1000.00
ED_{50} =	>50.00	134.89	229.08	281.83
Regression constants : Y = mx + c				
m =		89.909	87.463	82.992
c =		- 142.316	- 156.828	- 154.042
r =		0.999	0.999	0.999

* Data used for regression analysis.

Table - 9E

Effect of BD - 15 on growth of Alternaria solani

Concentration (µg/ml)	Percentage of inhibition over control after			
	24 hours	48 hours	72 hours	96 hours
500	100	100	98.71*	82.53*
400	100	97.40*	81.63*	74.90*
300	100	86.44*	70.55*	65.25*
250	100	80.25*	66.30*	55.60*
200	100	70.27*	53.30*	46.00*
150	100	53.75*	46.25*	34.25*
100	85.00	44.50*	34.25*	20.75*
50	62.35	36.15	18.50	8.30
ED_{95} =	>100.00	363.07	575.43	645.65
ED_{50} =	<50.00	114.81	158.48	208.92
Regression constants : Y = mx + c				
m =		69.440	79.635	91.406
c =		-134.716	- 125.126	- 162.556
r		0.999	0.999	0.998

* Data used for regression analysis.

Table - 9F

Effect of ED - 16 on growth of *Alternaria solani*

Concentration (g/ml)	Percentage of inhibition over control after			
	24 hours	48 hours	72 hours	96 hours
500	100	100	100	88.75*
400	100	100	91.70*	81.25*
300	100	90.00*	80.64*	70.00*
250	100	85.00*	73.80*	64.00*
200	100	77.50*	66.25*	56.15*
150	100	69.50*	55.00*	44.20*
100	80.00	52.50*	40.45*	30.62*
50	56.50	28.00	19.15	11.45
ED_{95} =	>100.00	323.59	426.57	575.43
ED_{50} =	< 50.00	89.12	128.82	169.82
Regression constants : Y = mx + c				
m =		80.583	85.420	84.645
c =		-107.783	- 130.344	- 138.884
r =		0.998	0.999	0.999

* Data used for regression analysis.

Table - 90

Effect of ED - 17 on growth of Alternaria solani

Concentration (µg/ml)	Percentage of inhibition over control after			
	24 hours	48 hours	72 hours	96 hours
500	100	100	76.66*	71.18*
400	100	100	68.00*	62.25*
300	100	71.05*	55.00*	49.15*
250	100	63.52*	47.50*	42.98*
200	100	55.36*	38.66*	33.89*
150	66.66	42.10*	28.50*	23.50*
100	50.00	26.31*	22.50	16.55
50	26.50	15.10	9.75	5.00
ED_{95} =	>150.00	512.86	776.24	891.25
ED_{50} =	100.00	173.78	257.03	288.40
Regression constants : Y = mx + c				
m =		95.662	93.840	92.066
c =		-165.102	- 176.280	- 177.176
r =		0.999	0.999	0.999

* Data used for regression analysis.

Table - XI

Effect of ED - 18 on growth of *Alternaria solani*

Concentration (μg/ml)	Percentage of inhibition over control after			
	24 hours	48 hours	72 hours	96 hours
500	100	100	89.13*	77.90*
400	100	92.50*	79.50*	69.50*
300	100	81.48*	67.50*	55.38*
250	100	75.70*	58.50*	47.60*
200	100	66.90*	51.08*	44.61*
150	75.00	55.55*	39.13*	30.76*
100	62.50	44.44*	26.78*	20.00
50	50.00	18.50*	13.50	9.50
ED_{95} =	>150.00	245.47	588.84	776.24
ED_{50} =	50.00	77.62	186.20	239.88
Regression constants : Y = mx + c				
m =		80.981	91.119	89.203
c =		- 118.552	- 157.540	- 163.051
r =		0.999	0.997	0.994

* Data used for regression analysis.

Table - 91

Effect of Hinosamon growth of Alternaria solani

Concentration ($\mu g/ml$)	Percentage of inhibition over control after			
	24 hours	48 hours	72 hours	96 hours
500	100	100	100	95.83*
400	100	100	100	90.62*
300	100	100	90.05*	74.50*
250	100	100	87.50*	68.33*
200	100	100	77.15*	57.08*
150	100	86.81*	63.68*	45.70*
100	80.00	70.83*	48.44*	20.50*
50	60.00	40.15*	17.55*	10.25
ED_{95} =	>100.00	177.82	309.02	446.68
ED_{50} =	< 50.00	60.25	104.71	169.82
Regression constants:	Y = mx + c			
m =		97.423	95.599	108.434
c =		- 124.369	- 143.390	- 192.800
r =		0.999	0.998	0.995

* Data used for regression analysis.

Table - 10A

Effect of BD - 10 on growth of Helminthosporium oryzae.

Concentration (µg/ml)	Percentage of inhibition over control after			
	24 hours	48 hours	72 hours	96 hours
500	100	100	91.50*	87.70*
400	100	100	77.50*	76.50*
300	100	76.74*	67.50*	65.81*
250	100	72.09*	62.50*	58.97
200	100	65.11*	52.50*	50.42*
150	100	56.50*	40.00*	35.04*
100	85.71	41.50*	27.50*	24.78*
50	57.14	18.60*	17.50*	11.11*
ED_{95}	> 100.00	500.00	575.43	602.55
ED_{50}	< 50.00	125.89	181.97	194.98
Regression constents : Y = mx + c				
m =		75.646	90.563	92.127
c =		-109.063	- 155.167	- 161.706
r =		0.999	0.995	0.996

* Data used for regression analysis.

Table - 10B

Effect of BD - 11 on growth of Helminthosporium oryzae.

Concentration ($\mu g/ml$)	Percentage of inhibition over control after			
	24 hours	48 hours	72 hours	96 hours
500	100	72.41*	63.26*	53.84*
400	100	62.05*	54.05*	52.30*
300	80.76*	53.44*	44.89*	43.07*
250	69.23*	44.82*	40.50*	33.77*
200	62.50*	36.50*	26.53*	26.15*
150	51.50*	27.50*	18.36*	16.92*
100	38.46*	17.24*	14.28*	12.30*
50	26.50*	13.79*	10.20*	9.25*
ED_{95} =	562.34	977.23	1122.01	1548.81
ED_{50} =	123.02	369.16	338.84	389.04
Regression constants : $Y = mx + c$				
m =	68.319	80.328	86.680	74.605
c =	- 93.429	- 145.87	- 169.942	-143.279
r =	0.978	0.995	0.992	0.980

* Data used for regression analysis.

Table - 10C

Effect of BD - 13 on growth of Helminthosporium oryzae

Concentration (μg/ml)	Percentage of inhibition over control after			
	24 hours	48 hours	72 hours	96 hours
500	100	80.42*	74.15*	67.25*
400	100	71.35*	66.15*	59.37*
300	100	58.92*	53.19*	47.50*
250	100	53.57*	45.74*	39.00*
200	100	45.50*	37.23*	30.25*
150	54.85	33.50*	27.65*	20.15*
100	45.50	18.50*	12.85*	9.25
50	36.15	11.25	8.07	3.15
ED_{95} =	>150	724.43	851.13	954.99
ED_{50} =	>100	223.87	263.02	316.22
Regression constants: Y = mx + c				
m =		88.746	88.447	92.368
c =		- 159.028	-164.848	- 181.150
r =		0.999	0.999	0.999

* Data used for regression analysis.

Table - 10D

Effect of BD - 14 on growth of Helminthosporium oryzae.

Concentration (µg/ml)	Percentage of inhibition over control after			
	24 hours	48 hours	72 hours	96 hours
500	100	100	100	100
400	100	100	92.00*	81.50*
300	100	100	82.15*	60.50*
250	100	92.50*	77.50*	59.65*
200	100	82.25*	65.50*	51.50*
150	100	74.15*	53.75*	42.65*
100	68.25	56.20*	38.15*	26.75*
50	53.10	28.90*	16.05	7.90
ED_{95} =	>100.00	263.02	407.38	630.95
ED_{50} =	< 50.00	83.17	131.82	186.20
Regression constants : Y = mx + c				
m =		90.755	91.937	84.581
c =		- 124.770	- 145.273	- 142.612
r =		0.998	0.996	0.983

* Data used for regression analysis.

Table - 10E

Effect of ED - 15 on growth of Helminthosporium oryzae

Concentration (µg/ml)	Percentage of inhibition over control after			
	24 hours	48 hours	72 hours	96 hours
500	100	100	90.42*	84.92*
400	100	86.38*	82.14*	76.77*
300	100	75.27*	72.61*	63.25*
250	100	67.50*	63.50*	56.34*
200	100	59.05*	55.90*	47.50*
150	85.00	48.81*	42.15*	35.44*
100	71.15	31.25*	26.50*	20.02*
50	49.96	18.15	11.50	8.25
ED_{95} =	>150.00	500.00	524.80	616.59
ED_{50} =	>50.00	154.88	173.78	208.92
Regression constants: Y = mx + c				
m =		-91.222	-93.136	-94.469
c =		-150.415	-159.243	-169.407
r =		0.999	0.998	0.999

* Data used for regression analysis.

Table - 10F

Effect of BD - 16 on growth of Helminthosporium oryzae.

Concentration (μ g/ml)	Percentage of inhibition over control after			
	24 hours	48 hours	72 hours	96 hours
500	100	89.09*	86.95*	78.51*
400	100	87.27*	81.52*	73.55*
300	100	78.50*	71.73*	65.28*
250	100	70.90*	63.04*	57.02*
200	100	64.50*	56.52*	48.76*
150	100	58.90*	48.50*	36.36*
100	76.20	45.45*	34.78*	27.27*
50	52.15	25.45*	21.25	15.15
ED_{95} =	>100.00	549.54	602.55	758.57
ED_{50} =	<50.00	114.81	158.48	199.52
Regression constants: Y = mx + c				
m =		65.628	76.528	78.519
c =		-85.198	-118.408	-131.178
r =		0.997	0.998	0.995

* Data used for regression analysis.

Table - 10G

Effect of ED - 17 on growth of Helminthosporium oryzae

Concentration (µg/ml)	Percentage of inhibition over control after			
	24 hours	48 hours	72 hours	96 hours
500	100	100	87.50*	77.96*
400	100	83.33*	78.75*	68.50*
300	100	68.50*	57.50*	55.93*
250	100	62.50*	50.00*	47.45*
200	100	52.75*	40.00*	38.98*
150	70.58*	39.50*	25.00*	23.72*
100	62.70*	23.50*	17.50*	14.10
50	41.17*	16.15	10.00	6.50
ED_{95} =	348.73	524.80	602.65	707.94
ED_{50} =	66.06	126.20	229.08	257.03
Regression constants: Y = mx + c				
m =	62.269	98.681	106.689	102.948
c =	- 63.483	- 174.025	- 202.417	-198.760
r =	0.995	0.999	0.986	0.999

* Data used for regression analysis.

Table - 10B

Effect of BD - 13 on growth of Helminthosporium oryzae

Concentration (µg/ml)	Percentage of inhibition over control after			
	24 hours	48 hours	72 hours	96 hours
500	100	78.90*	68.50*	62.50*
400	100	68.74*	59.61*	53.75*
300	68.42*	58.06*	47.50*	41.09*
250	60.90*	49.75*	38.46*	32.61*
200	50.75*	39.15*	30.75*	23.75*
150	36.50*	26.25*	17.07*	10.80*
100	20.57*	10.80*	8.75	5.25
50	14.42	6.65	5.50	0.00
ED_{95} =	524.80	707.94	891.25	1023.29
ED_{50} =	194.93	261.18	316.22	363.07
Regression constants: Y = mx + c				
m =	103.237	99.177	98.533	99.680
c =	- 186.508	-188.096	-196.445	-205.471
r =	0.999	0.999	0.999	0.999

* Data used for regression analysis.

Table - 101

Effect of Hinosan on growth of Helminthosporium oryzae.

Concentration (µg/ml)	Percentage of inhibition over control after			
	24 hours	48 hours	72 hours	96 hours
500	100	100	100	100
400	100	100	100	100
300	100	100	97.50*	91.17*
250	100	100	90.47*	86.76*
200	100	91.83*	83.33*	77.94*
150	88.50*	79.59*	73.80*	67.64*
100	75.00*	65.30*	59.52*	52.50*
50	56.00*	40.50*	29.50*	24.50*
ED_{95} =	186.20	218.77	269.15	309.02
ED_{50} =	39.81	63.09	81.28	93.32
Regression constants: Y = mx + c				
m =	66.924	83.583	86.126	86.554
c =	- 57.559	-101.205	- 114.540	-121.071
r =	0.997	0.999	0.998	0.999

* Data used for regression analysis.

Table - 11A

Effect of BD - 10 on spore germination inhibition of *Aspergillus niger*.

Concentration (µg/ml)	Percentage of inhibition over control after	
	24 hours	48 hours
	% inhibition	% inhibition
2000	100	64*
1600	71*	55*
1200	53*	39*
1000	44*	31*
800	31*	9
600	17*	0
400	0	0
ED_{95}=	2454.70	3630.78
ED_{50}=	1096.47	1445.43
Regression constants : Y = mx + c		
m =	125.999	111.737
c =	-333.285	-303.884
r =	0.998	0.998

* Data used for regression analysis.

Table - 11B

Effect of ED - 11 on spore germination of Aspergillus niger

Concentration (µg/ml)	Percentage of inhibition over control after	
	24 hours	48 hours
	% Inhibition	% Inhibition
2000	93*	74*
1600	70*	57*
1200	45*	35*
1000	26*	0
800	7	0
600	0	0
400	0	0
ED_{95} =	1995.26	2630.26
ED_{50} =	1258.92	1412.53
Regression constants : Y = mx + c		
m =	218.071	169.548
c =	-626.790	-485.527
r =	0.998	0.999

* Data used for regression analysis.

Table - 11C

Effect of BD - 13 on spore germination inhibition of Aspergillus niger

Concentration (μ g/ml)	Percentage of inhibition over control after	
	24 hours	48 hours
	% Inhibition	% Inhibition
2000	90*	75*
1600	78*	64*
1200	66*	46*
1000	60*	37*
800	49*	26*
600	37*	0
400	23*	0
ED_{95} =	2290.86	2818.38
ED_{50} =	776.24	1230.26
Regression constants :	Y = mx + c	
m =	95.502	125.178
c =	-226.752	-337.702
r =	0.998	0.999

* Data used for regression analysis.

Table - 11D

Effect of ED - 14 on spore germination inhibition of Aspergillus niger

Concentration (μg/ml)	Percentage of inhibition over control after	
	24 hours	48 hours
	% Inhibition	% Inhibition
2000	87*	75*
1600	78*	63*
1200	66*	49*
1000	60*	40*
800	49*	31*
600	37*	0
400	23*	0
ED_{95} =	2398.83	3019.95
ED_{50} =	794.32	1174.89
Regression constants: Y = mx + c		
m =	92.767	110.980
c =	-219.038	-201.775
r =	0.999	0.998

* Data used for regression analysis.

Table - 11E

Effect of ED - 15 on spore germination inhibition of Aspergillus niger.

Concentration (µg/ml)	Percentage of inhibition over control after	
	24 hours	48 hours
	% Inhibition	% Inhibition
2000	100	77*
1600	85*	61*
1200	69*	43*
1000	58*	37*
800	44*	23*
600	25*	0
400	0	0
ED_{95} =	1319.70	2754.22
ED_{50} =	870.96	1230.26
Regression constants : Y = mx + c		
m =	140.567	131.175
c =	-363.816	-356.45
r =	0.999	0.995

* Data used for regression analysis.

Table - 11F

Effect of BD - 16 on spore germination inhibition of Aspergillus niger

Concentration (µg/ml)	Percentage of inhibition over control after	
	24 hours	48 hours
	% Inhibition	% Inhibition
2000	65*	52*
1600	50*	40*
1200	33*	21*
1000	21*	11*
800	0	.0
600	0	0
400	0	0
ED_{95} =	3162.27	3981.07
ED_{50} =	1548.81	1905.46
Regression constants : $Y = mx + c$		
m =	143.968	137.866
c =	-410.170	-402.246
r =	0.998	0.999

* Data used for regression analysis.

Table - 11G

Effect of BD - 17 on spore germination inhibition of Aspergillus niger.

Concentration (μ g/ml)	Percentage of inhibition over control after	
	24 hours	48 hours
	% Inhibition	% Inhibition
2000	100	100
1600	100	81*
1200	76*	62*
1000	62*	49*
800	47*	33*
600	21*	12*
400	0	0
ED_{95}	1479.10	1905.46
ED_{50}	831.76	1000.00
Regression constants : Y = mx + c		
m =	180.353	161.785
c =	-477.837	-436.015
r =	0.998	0.999

* Data used for regression analysis.

Table - 11H

Effect of ED - 18 on spore germination inhibition of Aspergillus niger.

Concentration (µg/ml)	Percentage of inhibition over control after	
	24 hours	48 hours
	% Inhibition	% Inhibition
2000	78*	69*
1600	69*	58*
1200	54*	44*
1000	44*	34*
800	33*	26*
600	21*	0
400	0	0
ED_{95} =	2754.22	3388.44
ED_{50} =	1096.47	1318.25
Regression constants : Y = mx + c		
m =	110.998	109.868
c =	-287.603	-293.734
r =	0.998	0.997

* Data used for regression analysis.

Table - III

Effect of Hinosan on spore germination inhibition of Aspergillus niger.

Concentration (μg/ml)	Percentage of inhibition over control after	
	24 hours	48 hours
	% Inhibition	% Inhibition
2000	75*	53*
1600	66*	42*
1200	53*	29*
1000	47*	22*
800	41*	0
600	28*	0
400	11*	0
ED_{95} =	3311.31	5123.61
ED_{50}	1047.12	1362.08
Regression constants : Y = mx + c		
m =	89.814	102.934
c =	-221.533	-286.971
r =	0.998	0.999

* Data used for regression analysis.

Table - 124

Effect of ED - 10 on spore germination inhibition of Penicillium sensoni.

Concentration (g/ml)	Percentage of inhibition over control after	
	24 hours	48 hours
	% Inhibition	% Inhibition
2000	100	81*
1600	88*	60*
1200	61*	43*
1000	54*	31*
800	41*	18*
600	23*	0
400	0	0
ED_{95} =	1862.08	2511.88
ED_{50} =	912.01	1288.24
Regression constants : Y = mx + c		
m =	146.469	154.964
c =	- 384.252	-432.859
r =	0.992	0.996

* Data used for regression analysis.

Table - 12B

Effect of BD - 11 on spore germination inhibition of Penicillium gensenl.

Concentration (μg/ml)	Percentage of inhibition over control after	
	24 hours	48 hours
	% Inhibition	% Inhibition
2000	68*	57*
1600	53*	39*
1200	33*	17*
1000	23*	4*
800	0	0
600	0	0
400	0	0
ED_{95} =	2951.20	3236.93
ED_{50} =	1513.56	1819.70
Regression constants : Y = mx + c		
m =	150.675	175.454
c =	- 429.247	-522.114
r =	0.999	0.999

* Data used for regression analysis.

Table - 120

Effect of BD - 13 on spore germination inhibition of Penicillium roseni.

Concentration (μ g/ml)	Percentage of inhibition over control after	
	24 hours	48 hours
	% Inhibition	% Inhibition
2000	100	100
1600	100	95*
1200	92*	71*
1000	79*	65*
800	66*	38*
600	45*	14*
400	18*	0
ED_{95} =	1230.26	1548.31
ED_{50} =	630.95	891.25
Regression constants ; Y = mx + c		
m =	155.601	190.907
c =	-386.264	-511.142
r =	0.999	0.993

* Data used for regression analysis.

Table - 122

Effect of BD - 14 on spore germination inhibition of Penicillium commune

Concentration (µg/ml)	Percentage of inhibition over control after	
	24 hours	48 hours
	% Inhibition	% Inhibition
2000	70*	53*
1600	55*	39*
1200	37*	24*
1000	23*	13*
800	9*	0
600	0	0
400	0	0
	0	0
ED_{95} =	2884.08	4168.59
ED_{50} =	1445.43	1862.08
Regression constants : $Y = mx + c$		
m =	153.435	130.367
c =	-435.928	-337.431
r =	0.998	0.998

* Data used for regression analysis.

Table - 12E

Effect of ED - 15 on spore germination inhibition of Penicillium geneni.

Concentration (μ g/ml)	Percentage of inhibition over control after	
	24 hours	48 hours
	% Inhibition	% Inhibition
2000	100	91*
1600	100	76*
1200	100	52*
1000	66*	41*
800	44*	32*
600	29*	28*
400	11*	8
	0	6
ED_{95} =	1778.27	2344.22
ED_{50} =	812.83	1023.29
Regression constants : Y = mx + c		
m =	132.757	125.929
c =	- 338.543	- 329.493
r =	0.984	0.968

* Data used for regression analysis.

Table - 12F

Effect of ED - 16 on spore germination inhibition of Penicillium gensenl.

Concentration (μg/ml)	Percentage of inhibition over control after	
	24 hours	48 hours
	% Inhibition	% Inhibition
2000	69*	51*
1600	55*	37*
1200	38*	15*
1000	31*	6*
800	17*	0
600	0	0
400	0	0
ED_{95} =	3162.27	3801.89
ED_{50} =	1412.53	1949.84
Regression constants : $Y = mx + c$		
m =	128.276	153.097
c =	-354.887	-453.858
r =	0.999	0.998

* Data used for regression analysis.

Table - 126

Effect of BD - 17 on spore germination inhibition of Penicillium gensenl.

Concentration (μg/ml)	Percentage of inhibition over control after	
	24 hours	48 hours
	% Inhibition	% Inhibition
2000	100	100
1600	100	77*
1200	77*	57*
1000	64*	43*
800	52*	32*
600	34*	17*
400	9	0
ED_{95} =	1584.89	2137.96
ED_{50} =	758.57	1023.29
Regression constants : $Y = mx + c$		
m =	139.980	140.567
c =	-354.062	-373.816
r =	0.997	0.998

* Data used for regression analysis.

Table - 12H

Effect of BD - 13 on spore germination inhibition of *Penicillium sensen*.

Concentration (µg/ml)	Percentage of inhibition over control after	
	24 hours	48 hours
	% Inhibition	% Inhibition
2000	100	70*
1600	71*	66*
1200	53*	34*
1000	42*	24*
800	32*	0
600	11*	0
400	0	0
ED_{95} =	2344.22	2630.26
ED_{50} =	1096.47	1412.53
Regression constents : Y = mx + c		
m =	137.168	166.464
c =	-368.059	-474.615
r =	0.998	0.972

* Data used for regression analysis.

Table - 12I

Effect of Hinosan on spore germination inhibition of Penicillium gensenl.

Concentration (µg/ml)	Percentage of inhibition over control after	
	24 hours	48 hours
	% Inhibition	% Inhibition
2000	100	100
1600	100	71*
1200	68*	47*
1000	53*	32*
800	39*	18*
600	29*	8*
400	11*	0
ED_{95} =	2157.76	2454.70
ED_{50} =	891.25	1202.26
Regression constants : Y = mx + c		
m =	116.083	148.244
c =	-250.060	-407.553
r =	0.986	0.983

* Data used for regression analysis

Table - 13A

Effect of BD - 10 on spore germination inhibition of Verticillium albo-atrum.

Concentration (µg/ml)	Percentage of inhibition over control after	
	24 hours	48 hours
	% Inhibition	% Inhibition
2000	57*	36*
1600	44*	24*
1200	31*	11*
1000	24*	0
800	13*	0
600	0	0
400	0	0
ED_{95} =	4570.88	6918.30
ED_{50} =	1237.80	2691.53
Regression constants : Y = mx + c		
m =	103.051	108.270
c =	-300.643	-321.716
r =	0.988	0.998

* Data used for regression analysis.

Table - 13B

Effect of BD - 11 on spore germination inhibition of Verticillium albo-atrum.

Concentration (μg/ml)	Percentage of inhibition over control after	
	24 hours	48 hours
	% Inhibition	% Inhibition
2000	100	78*
1600	69*	55*
1200	43*	29*
1000	31*	17*
800	18*	0
600	0	0
400	0	0
ED_{95} =	2290.86	2398.83
ED_{50} =	1230.86	1445.43
Regression constants : Y = mx + c		
m =	171.316	203.493
c =	-480.979	- 594.727
r =	0.994	0.998

* Data used for regression analysis.

Table - 13E

Effect of BD - 13 on spore germination inhibition of Verticillium albo-atrum.

Concentration (µg/ml)	Percentage of inhibition over control after	
	24 hours	48 hours
	% Inhibition	% Inhibition
2000	100	100
1600	100	85*
1200	88*	62*
1000	79*	49*
800	66*	37*
600	43*	14*
400	18*	
ED_{95} =	1258.92	1819.70
ED_{50} =	630.96	977.23
Regression constants : Y = mx + c		
m =	161.251	162.345
c =	-374.988	-435.689
r =	0.998	0.998

* Data used for regression analysis.

Table - 139

Effect of ED - 14 on spore germination inhibition of Verticillium albo-atrum.

Concentration (μ g/ml)	Percentage of inhibition over control after	
	24 hours	48 hours
	% Inhibition	% Inhibition
2000	100	71*
1600	95*	58*
1200	75*	44*
1000	67*	36*
800	50*	22*
600	32*	8*
400	0	0
ED_{95} =	1584.89	3162.27
ED_{50} =	776.24	1318.25
Regression analysis : $Y = mx + c$		
m =	146.787	110.916
c =	-374.200	-321.671
r =	0.999	0.999

* Data used for regression analysis.

Table - 13E

Effect of ED - 15 on spore germination inhibition of Verticillium albo-atrum.

Concentration (µg/ml)	Percentage of inhibition over control after	
	24 hours	48 hours
	% Inhibition	% Inhibition
2000	100	62*
1000	63*	47*
1200	48*	29*
1000	37*	20*
800	28*	8*
600	14*	0
400	0	0
ED_{95}=	3019.95	3548.13
ED_{50}=	1230.76	1621.81
Regression constants : Y = mx + c		
m =	114.120	135.285
c =	-302.991	-385.374
r =	0.997	0.999

* Data used for regression analysis.

Table - 13F

Effect of BD - 16 on spore germination inhibition of Verticillium albo-atrum.

Concentration (μg/ml)	Percentage of inhibition over control after	
	24 hours	48 hours
	% Inhibition	% Inhibition
2000	100	58*
1600	57*	39*
1200	41*	17*
1000	36*	0
800	22*	0
600	0	0
400	0	0
ED_{95} =	3388.44	3235.93
ED_{50} =	1342.96	1778.27
Regression constants : Y = mx + c		
m =	114.105	177.819
c =	-308.167	-529.244
r =	0.995	0.999

* Data used for regression analysis.

Table - 130

Effect of BD - 17 on spore germination inhibition of Verticillium albo-atrum

Concentration (µg/ml)	Percentage of inhibition over control after	
	24 hours	48 hours
	% Inhibition	% Inhibition
2000	88*	77*
1600	72*	60*
1200	57*	41*
1000	47*	29*
800	34*	14*
600	19*	0
400	0	0
ED_{95}	2290.86	2570.39
ED_{50}	1023.29	1348.96
Regression constants : Y = mx + c		
m =	129.330	156.830
c =	-340.331	-441.034
r =	0.998	0.999

* Data used for regression analysis.

Table - 13H

Effect of BD - 13 on spore germination inhibition of Verticillium albo-atrum.

Concentration (µg/ml)	Percentage of inhibition over control after	
	24 hours	48 hours
	% Inhibition	% Inhibition
2000	60*	50*
1600	45*	35*
1200	27*	19*
1000	19*	0
800	7*	0
600	0	0
400	0	0
ED_{95} =	3715.35	4265.74
ED_{50} =	1698.24	1995.26
Regression constants : Y = mx + c		
m =	132.426	134.210
c =	-378.127	-393.464
r =	0.998	0.998

* Data used for regression analysis.

Table - 13I

Effect of Hinosan on spore germination inhibition of Verticillium albo-atrum

Concentration (µg/ml)	Percentage of inhibition over control after	
	24 hours	48 hours
	% Inhibition	% Inhibition
2000	100	100
1600	100	63*
1200	96*	52*
1000	87*	44*
800	72*	37*
600	59*	26*
400	43*	0
ED_{95}	1174.89	3715.35
ED_{50}	467.73	1122.01
Regression constants : Y = mx + c		
m =	112.837	86.141
c =	-252.218	-212.989
r =	0.995	0.998

* Data used for regression analysis.

Table - 14A

Effect of BD - 10 on spore germination inhibition of Helminthosporium oryzae.

Concentration (μg/ml)	Percentage of inhibition over control after	
	24 hours	48 hours
	% Inhibition	% Inhibition
2000	100	100
1600	98*	89*
1200	75*	63*
1000	66*	49*
800	45*	26*
600	26*	7
400	0	0
ED_{95}	1513.56	1659.52
ED_{50}	812.83	1023.29

Regression constants : $Y = mx + c$

m =	168.939	200.966
c =	-442.790	-579.031
r =	0.993	0.999

* Data used for regression analysis.

Table - 143

Effect of BD - 11 on spore germination inhibition of Helminthosporium oryzae.

Concentration (μg/ml)	Percentage of inhibition over control after	
	24 hours	48 hours
	% Inhibition	% Inhibition
2000	100	98*
1600	88*	76*
1200	59*	40*
1000	44*	26*
800	26*	0
600	11	0
400	0	0
ED_{95}	1698.24	1905.46
ED_{50}	1047.12	1258.92
Regression constants : Y = mx + c		
m =	207.813	245.924
c =	-578.022	-712.817
r =	0.998	0.998

* Data used for regression analysis.

Table - 14C

Effect of ED - 13 on spore germination inhibition of Helminthosporium oryzae.

Concentration (µg/ml)	Percentage of inhibition over control after	
	24 hours	48 hours
	% Inhibition	% Inhibition
2000	100	84*
1600	75*	70*
1200	56*	41*
1000	42*	28*
800	27*	10*
600	11*	0
400	0	0
ED_{95}	2137.96	2187.76
ED_{50}	1071.51	1288.24
Regression constants : Y = mx + c		
m =	151.494	190.309
c =	-410.464	-542.218
r =	0.997	0.997

* Date used for regression analysis.

Table - 14D

Effect of BD - 14 on spore germination inhibition of Helminthosporium oryzae.

Concentration (μg/ml)	Percentage of inhibition over control after	
	24 hours	48 hours
	% Inhibition	% Inhibition
2000	100	100
1600	100	100
1200	92*	81*
1000	73*	63*
800	51*	45*
600	24*	16*
400	0	0
ED_{95}	1230.26	1380.38
ED_{50}	776.24	831.76
Regression constants : $Y = mx + c$		
m =	224.361	212.475
c =	-593.500	-572.365
r =	0.998	0.998

* Data used for regression analysis.

Table - 14E

Effect of ED - 15 on spore germination inhibition of Helminthosporium oryzae.

Concentration (µg/ml)	Percentage of inhibition over control after	
	24 hours	48 hours
	% Inhibition	% Inhibition
2000	100	76*
1600	72*	60*
1200	56*	43*
1000	44*	29*
800	32*	16*
600	17*	0
400	0	0
ED_{95}	2392.83	2630.26
ED_{50}	1071.51	1318.25
Regression constants : $X = mx + c$		
m =	129.464	150.456
c =	-342.610	-420.713
r =	0.998	0.998

* Data used for regression analysis.

Table - 14F

Effect of BD - 16 on spore germination inhibition of Helminthosporium oryzae

Concentration (µg/ml)	Percentage of inhibition over control after	
	24 hours	48 hours
	% Inhibition	% Inhibition
2000	100	76*
1600	69*	58*
1200	53*	34*
1000	40*	24*
800	31*	0
600	11*	0
400	0	0
ED_{95}	2454.71	2570.39
ED_{50}	1122.01	1412.53
Regression constants : Y = mx + c		
m =	133.806	176.500
c =	-359.013	-508.510
r =	0.996	0.999

* Data used for regression analysis.

Table - 146

Effect of BD-17 on spore germination inhibition of Helminthosporium oryzae.

Concentration (µg/ml)	Percentage of inhibition over control after	
	24 hours	48 hours
	% Inhibition	% Inhibition
2000	100	100
1600	100	100
1200	87*	63*
1000	73*	48*
800	56*	36*
600	25*	19*
400	0	0
ED_{95}	1258.92	2041.73
ED_{50}	758.57	977.23
Regression constants : Y = mx + c		
m =	204.420	142.043
c =	-539.723	-375.396
r =	0.997	0.993

* Data used for regression analysis.

Table - 14H

Effect of ED - 18 on spore germination inhibition of Helminthosporium oryzae.

Concentration (µg/ml)	Percentage of inhibition over control after	
	24 hours	48 hours
	% Inhibition	% Inhibition
2000	100	100
1600	100	83*
1200	65*	57*
1000	49*	45*
800	29*	20*
600	8*	0
400	0	0
ED_{95}	1698.24	1778.27
ED_{50}	977.23	1071.51
Regression constants : Y = mx ± c		
m =	189.096	207.184
c =	-517.247	-579.107
r =	0.997	0.997

* Data used for regression analysis.

Table - 14I

Effect of Hinosan on spore germination inhibition of Helminthosporium oryzae

Concentration (μg/ml)	Percentage of inhibition over control after	
	24 hours	48 hours
	% Inhibition	% Inhibition
2000	100	100
1600	100	100
1200	100	100
1000	97*	86*
800	88*	69*
600	72*	49*
400	57*	23*
ED_{95}	933.25	1148.15
ED_{50}	338.89	588.84
Regression constants : Y = mx + c		
m =	101.979	156.760
c =	-208.826	-384.922
r =	0.977	0.999

* Data used for regression analysis.