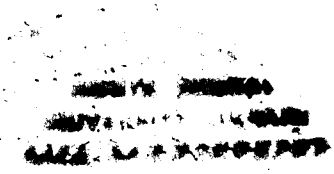
STUDIES ON (i) INSECTICIDAL, TOXICOLOGICAL AND OTHER BIOLOGICAL PROPERTIES OF SOME SALIGENIN CYCLIC PHOSPHORUS COMPOUNDS, AND (ii) CYTOLOGICAL EFFECTS OF SOME PESTICIDES ON PLANTS AND ANIMALS

Thesis Submitted for the Degree of
Doctor of Philosophy (Science)
of the
University of North Bengal



Sharmila Guha, M. Sc.

PESTICIDE RESEARCH LABORATORY
DEPARTMENT OF CHEMISTRY
UNIVERSITY OF NORTH BENGAL
1992

Dedicated

to my

Grand Parents

ACKNOWLEDGEMENT

Research works were presented in this dissertation, have carried out by me at the Pesticide Research Laboratory, Department of Chemistry, University of North Bengal, Raja Rammohunpur, District Darjeeling.

I take this opportunity to express my deep sense of gratitude and thankfulness to Dr. Bimal Kumar Das, D.Sc., Professor of Chemistry, University of North Bengal, for his continuous guidence, valuable suggestions, attention and encouragement during the progress of the work.

I wish to express my gratitude and indebtness to:

Prof. M. Eto, Department of Agricultural Chemistry, Kyushu University, Fukuoka, Japan, for helping me to get a number of reprints.

RSIC, Central Drug Research Institute, Lucknow for Mass and NMR spectra of the compounds.

Head of the Department of Mycology and Plant Pathology IARI, New Delhi for sending me the pure culture of fungi.

The Department of Environment and Forest, Govt. of India for awarding me a fellowship under Co-ordinated Eco-Development Centre (Sikkim) Programme through the University of North Bengal.

I am thankful to all teaching and non-teaching staff of the Department of Chemistry, Zoology and Botany of the University of North Bengal and Siliguri College.

I should be failing in my duties if I do not mention the active co-operation rendered by my fellow research scholars Dr. (Mrs.) J. Dasgupta, Miss S. Misra, Dr. B.K.Bhattacharya, Mr. D.B. Khawas, Dr. A.Samad, Dr. A. Lahiri, Dr. P. Roy, Dr. A. Adhikari, Mr. N. Sarma, Mrs. K. Pal, Mrs. P. Chakraborty, Mr. S. Pal and many other research workers, friends and good wishers.

I should like to thank Dr. S.Das, Electro Instrumentation Officer, Department of Chemistry, for excellent technical help.

Specific thanks to Mr. M. Chakraborty for typing the manuscript.

Finally I take this opportunity to record my sincere thanks to my parents and my sister, for their keen interest in my academic pursuit. They encouraged and inspired me all along.

Sharmila Guha

Date 6.3.92

(Sharmila Guha)

Pesticide Research Laboratory,
Department of Chemistry,
University of North Bengal,
Raja Rammohunpur-734430
District-Darjeeling
West Bengal.

C O N T E N T S

***

S Y N O P S I S

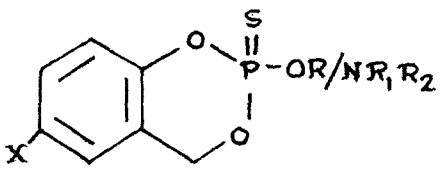
The work embodied in this dissertation is related to the investigation on some chloro/bromo/nitro saligenin cyclic phosphoramidothionates and phosphorothionates with reference to their synthesis, insecticidal activities, chemical hydrolysis, anticholinesterase activities, antifungal activities, phytotoxicity, acute oral and delayed neurotoxicity, cytotoxicity and other properties besides structure elucidations by chemical analysis and spectroscopic methods.

CHAPTER - 1 (Part I) of this thesis gives a brief introduction of saligenin cyclic phosphates describing the chemical, biochemical, insecticidal, fungicidal and other toxicological properties with special emphasis on salithion (2-methoxy-4H-1, 3,2-benzodioxaphosphorin-2-sulphide) discovered in 1963 by prof. Eto, Prof. Oshima and their co-workers of Kyushu University, Japan. Investigations have 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 and/or in hetero-cyclic ring. The biological activities of these compounds may be attributed to the hetero-ring involving enol and benzyl ester linkage. The alkylation reaction may be responsible for "SH-enzyme" inhibition and fungicidal activity. The phosphorylation reaction is responsible for esterase inhibition, animal toxicity and insecticidal activity. An exocyclic

substituent group affects physical and biological properties by virtue of its electronic and steric characteristics. Thus, methyl phosphorothionate is useful as an insecticide, alkylamidates have systemic activity, alkylphosphorothionates have fungicidal activity, phenyl phosphonates have antifilarial activity and aryl phosphates are neurotoxic and also have synergistic activity.

It was reported by Prof. Eto and his co-workers that 2-methoxy-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-sulphide (BD-8) was obtained as a paste after purification through silicic acid column chromatography and found to have about sixty times less insecticidal activity compared to salithion. However, it has been observed in this laboratory that the methoxy compound (BD-8) is a solid (m.p. 84°C) and has about 1.5-2 times greater oral insecticidal activity to Periplaneta americana (Lin.) than salithion (Das, B.K., D.Sc. Thesis, Calcutta University, 1981). These observations prompted us to undertake a systematic investigation on some 6-chloro/bromo/nitro saligenin cyclic phosphoramidothionates and phosphorothionates.

Aims and Objectives of these present investigation have been presented in CHAPTER - 1 (Part - II). The work presented in CHAPTER -2 (Part I and Part II) of this thesis is related to the investigation on some 2-alkylamido/alkoxy-6-chloro/bromo/nitro-4H-1,3,2-benzodioxaphosphorin-2-sulphide having the general structure (A).



(A)

Where, R_1/R_2 = Alkyl group / H

X = Cl, Br, NO_2

R = Alkyl / Phenyl.

The alkoxy groups are methoxy-ethoxy, isopropoxy and phenoxy; the alkylamido groups are N,N-diisobutyl amido, N,N-dibutyl amido, N-butyl amido and N-hexyl amido.

The above mentioned compounds have been prepared by condensation of the 5-chloro/bromo/nitro saligenin with the corresponding alkoxy/alkyl amido dichloridophosphorothionates, in presence of potassium carbonate which acts as a dehydrogen chloride agent. The N-hexylamido (BR-27) and chloro/bromo derivatives of N,N-dibutylamido (CL-10 and BR-10) compounds are liquid, and the rest are solid in nature.

The structure of these compounds have been determined by chemical analysis and UV, IR, Mass, NMR spectral data.

The common IR bands for the compounds are:

1000-1070 cm^{-1} (s)	P-O-C (alkyl)
1185-1260 cm^{-1} (s) and	
875-950 cm^{-1} (s)	P-O-C (aryl)
1515-1530 cm^{-1} (s)	asym. str. of nitro group
1340-1345 cm^{-1} (s)	sym. str. of nitro group
785-830 cm^{-1}	P = S (I)
645-665 cm^{-1}	P = S (II)

The compounds show parent molecular ion (M^+) peaks in the mass spectra. Fragmentation by loss of 'SH' radical is important. Compounds show an ion due to $(M - {}^{\cdot}SH)^+$, and it is the base-peak for some of the compound.

The experimental part on the biological and hydrolytic properties of the compounds have been presented in Appendix.

The isopropoxy compound (BD-5) shows about 1.5 times, the methoxy-ethoxy about 3.5-5 times greater insecticidal activity compared to Salithion, but in case of chloro derivative of N-butylamido compound (CL-24) the insecticidal activity is almost similar to that of Salithion. The other compounds are almost similar to that of Salithion. The other compounds are almost insecticidal. For Blow-fly and Grasshopper, the methoxy compound is more active than Salithion, the isopropoxy compound have some insecticidal activity, the methoxy-ethoxy compound also have some insecticidal activity on Grasshoppers. For Aphids the methoxy compound (BD-8) is the most active and phenoxy compound (BD-9) has least insecticidal activity.

Compared to Salithion all compounds are less toxic to rat. The isopropoxy compound (BD-5) and methoxy compound (BD-8) have greater toxicity (LD_{50}) in comparison to other compounds. The CL-6, BR-10, BR-24 and BR-27 showed some apparent toxic symptoms, some died within two months and the rest recovered soon.

The histopathological study of some organs of compound treated rats, showed some toxic effects. The N,N-diisobutyl amido compound (CL-6) was used for the tests. The section of kidney showed enlarged, mildly dialated tubules with ruptured walls. The renal tubules were elongated, enlarged lumen, ruptured epithelial lining. The glomerulus were disorganised and Bowman's capsules were very reduced. The section of liver showed the vacuoles, ruptured cell membranes, some cells without nuclei. The sections of intestine, lung and spleen showed no toxic effects.

Only four compounds (CL-6, BR-6, BD-5 and BD-8) have studied for acute oral toxicity and delayed neurotoxicity in hens. In those compounds BD-8 is the most toxic. Permanent paralysis in legs is observed only in case of N,N-diisobutyl amido compound (CL-6), upon histopathological examinations demyelination of sciatic nerve is found. In other compounds, permanent paralysis is not observed and the histopathological observation of sciatic nerves are normal.

Acetylcholinesterase inhibition data shows that the compound BD-1 (methoxy-ethoxy) has highest and CL-6 (N,N-diisobutyl amido) has lowest inhibitory activity for BFAChE. In case of goat whole blood, the anticholinesterase activity of the compound BD-25 (N,N-diisobutyl amido) is highest and BR-6 (N,N-diisobutyl amido) is lowest.

The antifungal activity study (by growth inhibition method) against H. oryzae and P. oryzae indicate that the chloro and bromo compounds show very good inhibitory effects on the growth of both the fungi H. oryzae and P. oryzae, their inhibitory effects are almost comparable to that of Ediphenphos (Hinosan).

The compounds are not phytotoxic to Triticum spp. up to the concentration 500 ppm.

The rate of hydrolysis of the compound is greatly influenced by the nature of the substituent at the 6-position of the benzodioxaphosphorin ring, it is observed that the 6-chloro saligenin cyclic phosphoramidothionates are most stable and 6-bromo saligenin cyclic phosphoramidothionates are least stable to alkaline hydrolysis.

The work presented in CHAPTER - 3 is the cytological effects of some Pesticides on Plants and Animals. The main effect after application of the compound N,N-diisobutyl amido (CL-6), Ediphenphos (Hinosan) and Rogor were found in anaphase stages of plant (root tips of onion) and the metaphase stage of animal (bone marrow cells of mice). In plants, different type of aberrations were observed viz. laggards, chromosome bridge, sticky chromosomes, tripolar and tetrapolar aberrations etc. The effect in animal cell was studied in metaphase stage of bone-marrow cells, the observation showed some aberrations, viz. formation of ring chromosome, breaks, gaps etc.

The biological activities and other data justify further examination of methoxy and isopropoxy compounds as potential insecticides and the chloro and bromo compounds as potential fungicides. Whether the use of these cyclic phosphorus compounds will protect the plants from pests and diseases in the field remains to be studied. In order to find out the chemical structure-biological activity relationship in these compounds we have to synthesize several new compounds in which different group is to be incorporated in different positions of the aromatic ring and to investigate their biological activity. Besides, structural eludication in regard to the conformation of the dioxaphosphorin ring from temperature dependent NMR and X-ray crystal structure would clarify the chemical structure-antifungal activity mechanism (and/or esterase inhibition mechanism) so that their selectivity of action can be known, thereby helping us to design selective and biodegradable potential pesticide.

CHAPTER - 1

PART - I

INTRODUCTION

One of the most important classes of pesticidal substances is the organophosphorus compounds. Several new compounds of this group possess insecticidal, acaricidal, nematocidal, anthelmintic, insect sterillizing, fungicidal, herbicidal, rodenticidal and other properties. The systemic investigations of their fungicidal and bactericidal properties were began after 1940. Owing to the wide diversity in their pesticidal activities, these organophosphorus compounds have won epoch-making popularity for use in field as potential pest controlling agents. Discovery of salithion cyclic phosphate as a biologically active metabolite of tri-o-cresyl phosphate (TOCP) has led to extensive studies on synthesis, chemical and biological properties of many related compounds.

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

Results on hens showed that neurotoxic triaryl phosphates, except tri-p-ethyl phenyl phosphate, have at least one alkyl group carrying the α-hydrogen atom on the ortho position[3,4].

This structure-neurotoxicity relationship of triaryl phosphates became clearly understandable by the isolation and characterisation of the active metabolites of TOCP in 1961[5,6]. The principal metabolite (A) was o-tolyl saligenin cyclic phosphate (2-o-tolyloxy-4H-1, 3,2-benzodioxaphosphorin 2-oxide). It is extraordinarily active in all the biological properties shown by TOCP : (A) was about 100 times more potent to cause ataxia in hens than TOCP, (A) was ten million times more active than TOCP in the <u>in vitro</u> inhibition of plasma-cholinesterase[7].

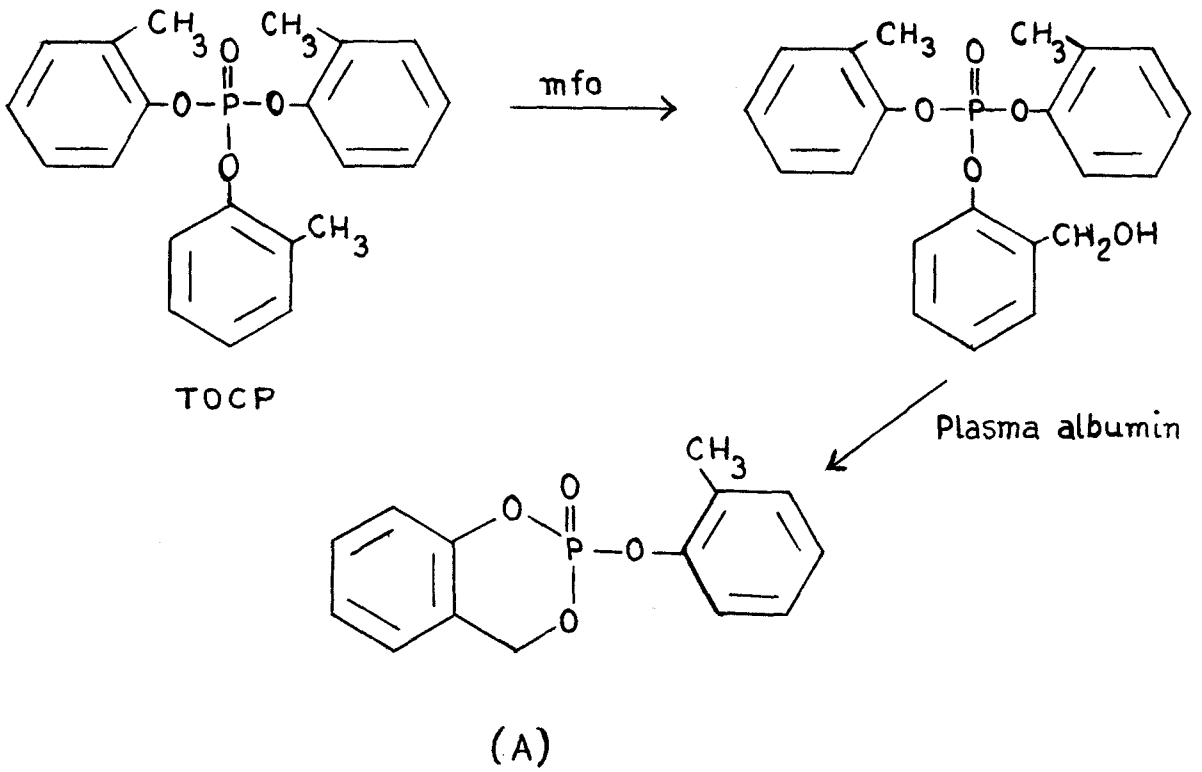
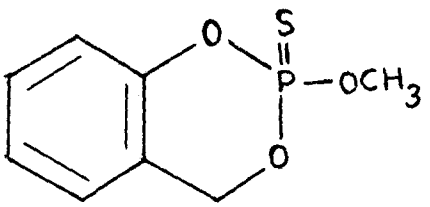


Fig. 1 : Metabolic activation of TOCP

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

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

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



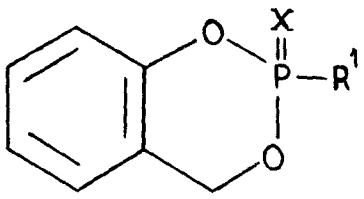
Salithion

Here we give an account of Salithion and related compounds as pesticides as well as their chemistry and biochemistry.

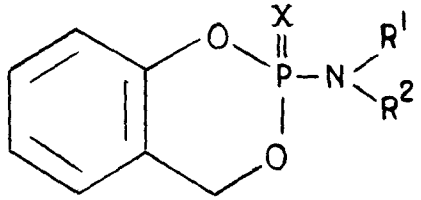
1. Synthesis of Saligenin cyclic phosphorus esters:

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

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



I



II

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

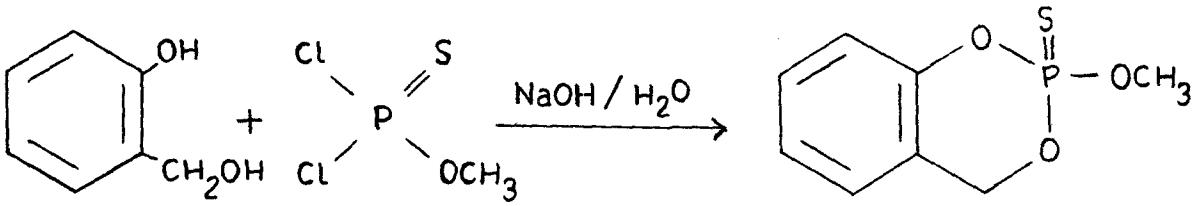


Fig. 2 Synthesis of Salithion

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

The improved process was carried out in an aqueous solution at a lower temperature and in a shorter reaction time period than the known process. Also, it gave an objective product

of better yield having higher purity than the known process. Salithion was obtained smoothly in a crystalline form (m.p. 49-53°C) under mild conditions (20°C, 2 hrs.) with a consistently high yield (70-80%)[16]. This method was applied also to synthesize other thiono analog of saligenin cyclic phosphorus esters from relatively less reactive dichlorides such as dialkyl phosphoramidodichloridothionates (formula-II).

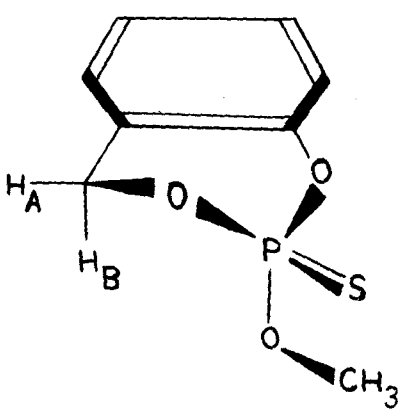
Further, it was possible in the Schotten-Baumann procedure to produce cyclic dithiophosphate esters such as S-alkyl cyclic phosphorothiolothionates of saligenin (i.e. X = S, R^1 = S-alkyl in formula - I) which cannot be produced at all by the known process.

2. Properties of Salithion:

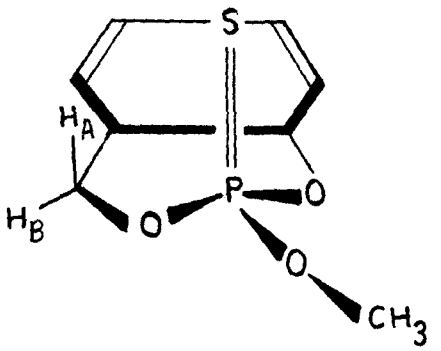
Referring back to Salithion, we pinpoint our discussion to its important properties[17] relating to its structure, degradation, isomerization etc.

Pure salithion is a colourless crystalline powder, m.p. 55-56°C, practically insoluble in water, easily soluble in acetone and benzene, moderately soluble in cyclohexane, toluene and xylene, vapour pressure 1.5×10^{-6} mm Hg at 25°C, UV λ_{max}^{nm} (ϵ) 274(860), 267(860). Salithion has a characteristic IR band at 1020 cm^{-1} for P-O-CH_2 in hetero ring. NMR δ(CS_2)ppm, 3.76 (3H, doublet, J_{pH} = 14Hm, CH_3), 5.21 (2H, doublet, J_{pH} = 15 Hm, CH_2), 6.8-7.2 (4H, multiplet, benzene ring).

The signal at the upper field of the doublet at 5.21 ppm slightly split further (1.5 Hz). This became significant at -30°C, suggesting that the methylene protons (H_A, H_B) were not equivalent to each other, but the dioxaphosphorin ring is conformationally mobile in a solution (Fig. 3). X-ray crystallographic analysis showed that the hetero ring of Salithion was a half-chair form in which the sulphide group was in equatorial position (III). The strain in the ring appeared little, the endocyclic O-P-O angle was 104°.



III



IV

Fig. 3 Conformational change of Salithion hetero-ring

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

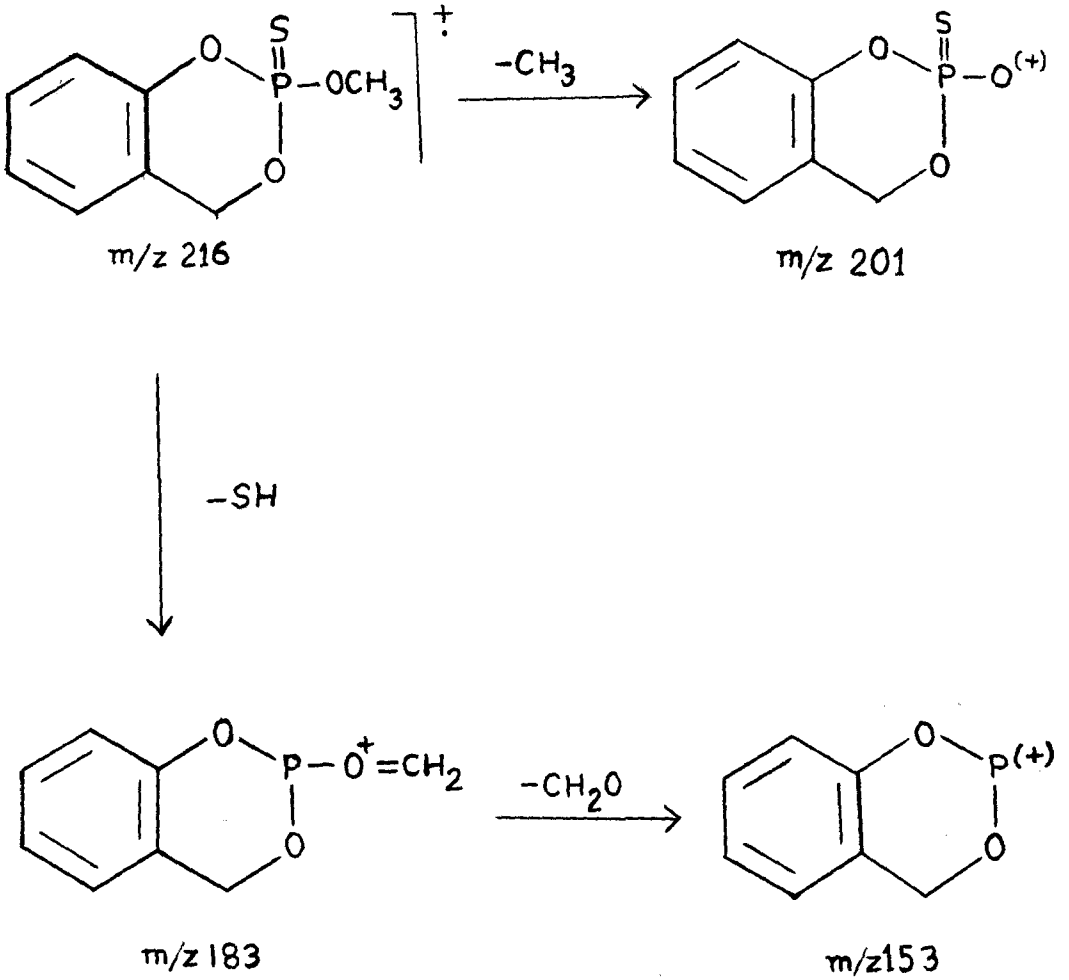


Fig. 4 Fragmentation of Salithion in Mass spectrometry.

Salithion is relatively unstable in storage. Some secondary amines, such as carbazole and N-phenyl-∝-naphthyl amine, stabilize the formulation[18]. In a phosphate buffer (pH 7.7) salithion was hydrolysed slowly through opening of the hetero ring by the P-O-(aryl) bond cleavage ; the hydrolysis rate constant (25°C) K = 2.4 x 10^{-4} min^{-1}. The hydrolysis rates of the corresponding cyclic methyl phosphate, S-methylphosphorothiolate (the thiolate isomer of salithion, MTBO), methyl phosphate (Salioxon), and N-methyl phosphoramidate were, respectively, 90, 60, 6 and 0.6 times more than that of salithion.

Salithion was completely hydrolysed by heating at 100°C for 5 min. with N/6 sodium hydroxide to yield saligenin. This was applied for the colorimetric determination of salithion in formulations by allowing the formed saligenin to react, after adjusting pH 8, with 4-aminoantipyrine and then with potassium ferricyanide[19,20].

On oxidation by bromine water salithion was converted to its oxon (salioxon). It was found that the cholinesterase inhibition of salioxon (2-methoxy-4H-1,3,2-benzodioxaphosphorin-2-oxide) was some thousand times more active than salithion, an enzymatic method after the oxidation was used for the residue analysis of salithion[19].

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

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

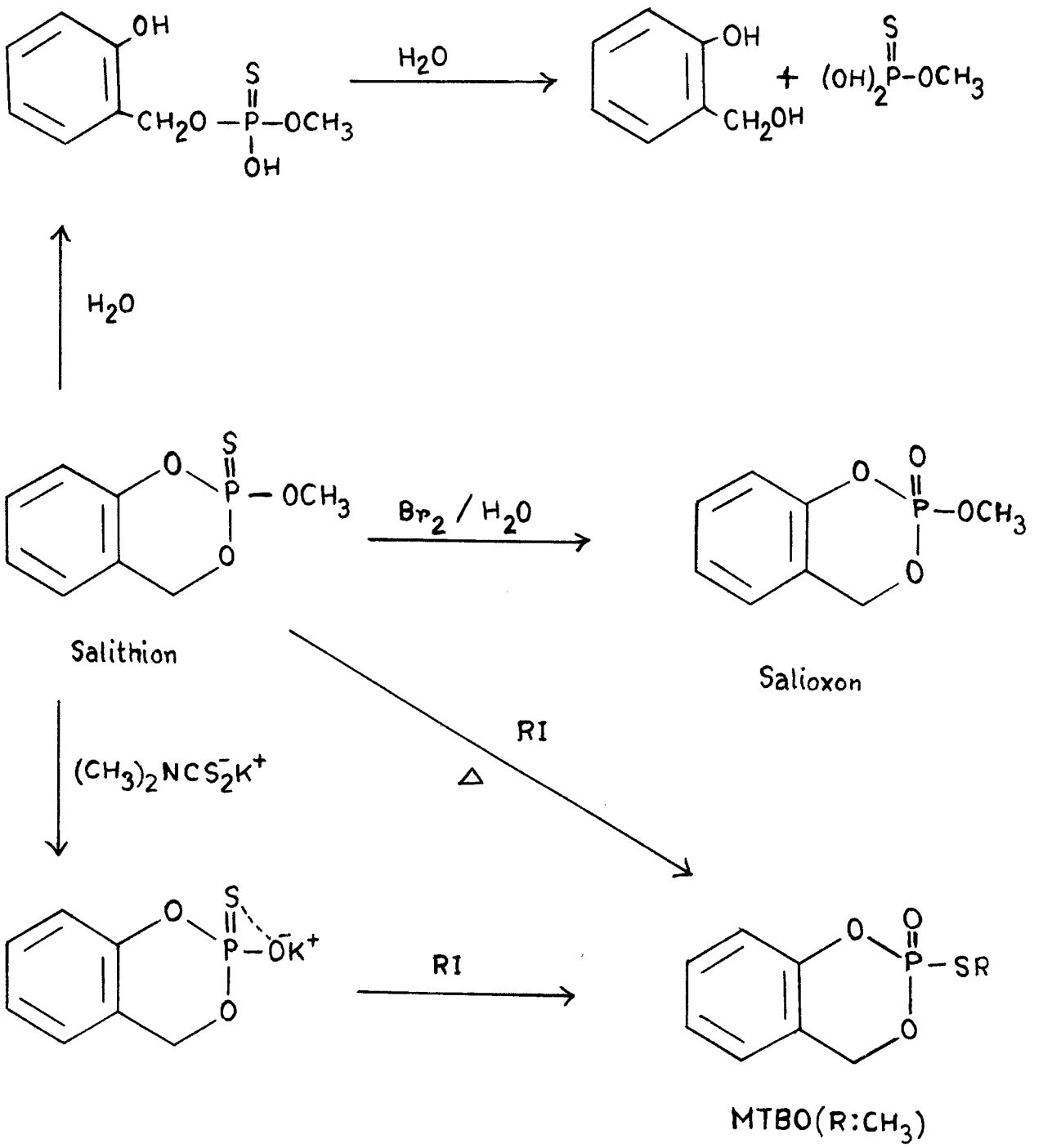


Fig. 5 Reaction of Salithion.

Salithion was a broad-spectrum insecticide for use in orchards and vegetable gardens. It was particularly effective to control lepidopteran larvae, mealybugs, aphids and mites. It exhibited the insecticidal action not only as contact and stomach poisons but also as a fumigent[17]. The residual toxicity of Salithion was so small that a natural enemy, Pseudaphycus malinus, could be used co-operatively for the control of comstock mealybug[8].

Acute toxicity to mammals was moderate. LD_{50} in mice by oral administration was 91.3 mg/kg, for male rats 52-125 mg/kg for female rats 102-180 mg/kg, for hens 110 mg/kg. Salithion 32P applied topically to houseflies was rapidly absorbed in the body (42% after 1 hr.). The major part was degraded in the body and about 4% of applied or 10% of absorbed Salithion, remained as Salithion and Salioxon for 24 hrs. On the other hand, Salithion 32P administered orally to rat was rapidly degraded and excreted.

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

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

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

Chronic toxicity tests[17] revealed that the rats fed for 24 months with 10 ppm Salithion showed slight decrease in cholinesterase activities. No effect was however, observed in rats fed with 3 ppm Salithion. No histological lesion was found in any organs of rats fed with 100 ppm.

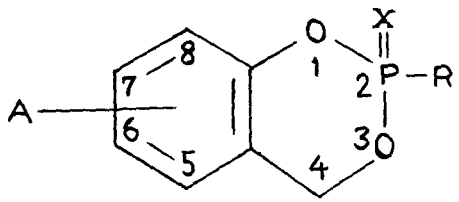
In men and women, administered orally 0.02 mg/kg/day of Salithion for 21 days followed by 0.05 mg/kg/day for 14 days, no effect was found in the activity of erythrocyte acetyl-cholinesterase. Carcinogenicity was not observed. No effect was observed in fertility of rats for three generations fed with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

3. Other Saligenin cyclic phosphorus esters:

A survey of literature furnishes[14,15,16,24,25,26,27] a variety of Saligenin cyclic phosphorus esters in good number, which had been prepared and examined for insecticidal activity. They involve phosphates, phosphorothiolates, phosphoramidates, phosphonates and their thiono-analogs . A comprehensive but not a complete list of Saligenin and ring-substituted Saligenin cyclic phosphorus esters are given in Table - 1.

Table - I

Substituted Saligenin Cyclic phosphorus esters with Physical Properties



Code No.	R	A	X	Procedure*	b.p. °C/mm Hg (m.p. °C)
K-7	OCH_3	H	O	(P)	110~2°/0.05
K-13	O-n-C_3H_7	H	O	(P)	129-32°/0.05
K-18	O-n-C_4H_9	H	O	(P)	150-4°/0.05
K-8	OC_2H_5	H	S	(P)	Liquid (not distilled)
K-16	OC_6H_5	H	S	(P)	(36°)
K-15	C_6H_5	H	S	(P)	(37°)
	CH_3	H	O	(P)	140°/0.5 (35°)
	C_2H_5	H	O	(P)	143~9°/0.3 (25°)
	i-C_3H_7	H	O	(P)	(80°)
	Sec-C_4H_9	H	O	(P)	110°/0.5
	t-C_4H_9	H	O	(P)	(74°)
	$CH = CH_2$	H	O	(P)	155°/2.5
	CH_2Cl	H	O	(P)	160/0.8 (51°)
	CH_2CH_2Cl	H	O	(P)	139-141/0.1
	CH_3	H	S	(P)	130/0.6
	C_2H_5	H	S	(P)	120/0.6
	i-C_3H_7	H	S	(P)	108/0.6
	CH_2Cl	H	S	(P)	146-155/0.4

Contd..

Code No.	R	A	X	Procedure*	b.p. °C/mm Hg (m.p. °C)
	OCH_3	6-CH_3	O	(P)	136-140/0.3
	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)	187/0.18
	OC_6H_5	6-Cl	O	(P)	(89°)
	$NHCH_3$	6-Cl	O	(P)	(148°)
	OCH_3	8-Cl	O	(P)	170-171/0.15
	OC_2H_5	8-Cl	O	(P)	151/0.18
	O-n-C_3H_7	8-Cl	O	(P)	183/0.18
	O-i-C_3H_7	8-Cl	O	(P)	137/0.04
	OC_6H_5	8-Cl	O	(P)	203/0.52 (54°)
	$NHCH_3$	8-Cl	O	(P)	(128-129°)
	OCH_3	6-CH_3	O	(P)	(34-35°)

Contd..

Code No.	R	A	X	Procedure*	b.p. °C/mm Hg (m.p. °C)
	OC_2H_5	6-CH_3	O	(P)	(71-72°)
	O-n-C_3H_7	6-CH_3	O	(P)	158-160/0.2
	OCH_3	7-CH_3	O	(P)	110-115/0.65
	OC_2H_5	7-CH_3	S	(S)	125-130/0.65
	O-n-C_3H_7	7-CH_3	S	(S)	140-142/0.65
	OCH_3	8-CH_3	S	(S)	68-70/0.15
	OC_2H_5	8-CH_3	S	(S)	108-109/0.15
	O-n-C_3H_7	8-CH_3	S	(S)	120-124/0.15
	$NHCH_3$	8-CH_3	S	(S)	(30°)
	OCH_3	6-C_6H_5	S	(S)	Oil**
	OC_2H_5	6-C_6H_5	S	(S)	Oil**
	O-n-C_3H_7	6-C_6H_5	S	(S)	Oil**
	OCH_3	6-OCH_3	S	(S)	Paste**
	OCH_3	6-$COCH_3$	S	(S)	Paste**
	OCH_3	6-Cl	S	(P)	170-178/0.2
	OCH_3	6-Cl	S	(P)	175-180/0.25
	$NHCH_3$	6-Cl	S	(S)	160-170/0.2
	SCH_3	6-Cl	S	(S,P)	(72-73°)
	OCH_3	8-Cl	S	(P)	(46-47°)
	$NHCH_3$	8-Cl	S	(S)	Oil**
	SCH_3	8-Cl	S	(S)	Paste**
	OCH_3	6-NO_2	S	(S)	Paste**
	OCH_3	6-Cl, 8-C_6H_5	S	(S)	Paste**
	OC_2H_5	6-Cl	S	(S)	Paste**
	O-n-C_3H_7	"	S	(S)	Paste**

Contd..

Code No.	R	A	X	Procedure*	b.p. °C/mm Hg (m.p. °C)
	OCH_3	{ 6-C_6H_5 8-Cl	S	(S)	Paste**
	OC_2H_5	"	S	(S)	Paste**
	O-n-C_3H_7	"	S	(S)	Paste**
	OCH_3	6,8-Cl	S	(S)	(57-58°)
	OC_2H_5	"	S	(S)	Oil**
	$NHCH_3$	"	S	(S)	Oil**
	SCH_3	H	S	(S)	(69-70)
	SC_2H_5	H	S	(S)	145-147/0.2
	S-n-C_3H_7	H	S	(S)	145-150/0.25
	S-i-C_3H_7	H	S	(S)	140-143/0.1
	S-C_3H_5	H	S	(S)	140-147/0.3
	S-n-C_4H_9	H	S	(S)	160-167/0.25
	S-C_6H_5	H	S	(S)	(79-80)
	SCH_3	H	O	(P)	144-5/0.1
	SC_2H_5	H	O	(P)	140-5/0.04
	S-n-C_3H_7	H	O	(P)	145-7/0.07
	S-i-C_3H_7	H	O	(P)	155-8/0.1
	S-n-C_4H_9	H	O	(P)	157-60/0.02
	SC_6H_5	H	O	(P)	(88-9)
	$NHCH_3$	H	O	(triethylamine)	(87°)
	NHC_2H_5	H	O	(P)	(68°)
	$N(CH_3)_2$	H	O	(triethylamine)	(121°)

Contd..

Code No.	R	A	X	Procedure*	b.p. °C/mm Hg (m.p. °C)
	$N(C_2H_5)_2$	H	O	(Potassium carbonate)	133-6/0.5
	$NHCH_3$	H	S	(P)	120-3/0.2
	NHC_2H_5	H	S	(Potassium carbonate)	Undistilled liquid
	$N(CH_3)_2$	H	S	(Potassium carbonate)	118-22/0.2
	$N(C_2H_5)_2$	H	S	(Potassium carbonate)	110/0.2

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

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

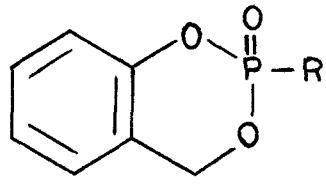
4. Structure and Specificity in Biological Activities:

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

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

Table - 2

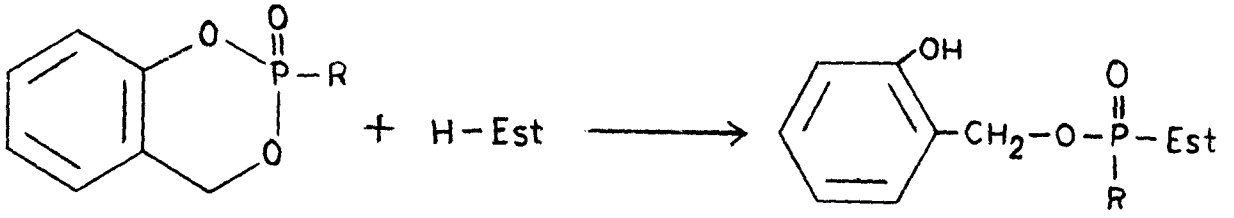
Effect of the exocyclic substituent (R) on biological activities of Saligenin cyclic phosphates



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

a. Minimum dose for causing ataxia in 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.

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



V VI

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

The ester became a more selective inhibitor of aliesterases[30] when the size of exocyclic substituent (R in V) increased, whereas it became a more selective inhibitor of cholinesterase when the substituent was small. Thus, the O-tolyl derivative (8), for example, inhibited aliesterase 130 times more than cholinesterase. Therefore, the exocyclic substituent of Saligenin cyclic Phosphate esters were regarded

as the selectophore in biological actions.

The heterocyclic structure of Saligenin cyclic phosphorus esters did not contribute towards the delayed neurotoxicity, but it merely induced the chemical reactivity of the Phosphorus atom for nucleophiles including the active site of esterases.

The nervous tissues, which Johnson found "neurotoxic esterase" were specifically sensitive in vivo to neurotoxic organophosphorus esters[31]. The esterase were unlike acetylcholinesterase but similar to chymotrypsin and trypsin in structure activity relationship of inhibitors[32]. Although the structure-neurotoxicity relationship were too complicated to be generalised, the neurotoxicity appeared to relate more with the structure of non-leaving groups than that of the leaving one. Neurotoxic esterase were remarkably resistant to most of the methyl esters[33].

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

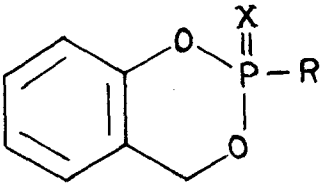
5. Insecticidal activity:

As stated earlier, the cyclic esters in any particular series having a small alkyl group have high insecticidal activity[8] (Table - 3), the Methyl derivatives were much more active than higher alkyl and aryl derivatives, except for

phosphate series in which the ethyl derivatives were more active[24] than the methyl one. N,N-Dialkyl phosphoramidates are much less active than mono-alkyl derivatives. Thus, Saligenin cyclic methyl phosphate, methyl phosphorothionate, N-methyl phosphoramidate, N-methyl phosphoramidothionate, methyl phosphorothiolate and ethyl phosphonothionate are potent insecticides. It is interesting to note that the exocyclic substituents of the most active cyclic phosphorus esters (OCH_3, $NHCH_3$, CH_2CH_3, SCH_3) differ from each other in electronic characteristics, but resembles in steric property such as the distance (about 2.9Å°) between phosphorus and carbon atoms in the P-X-C function, if supposing the bond angle of divalent sulphur near 90° rather than 109.5°[17].

Table - 3

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



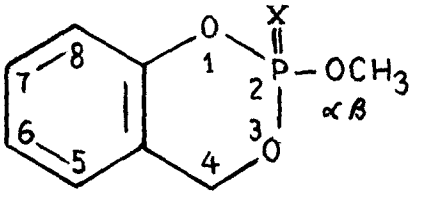
R	X		R	X	
	O	S		O	S
CH_3	0.13	0.31	CH_3S	0.09	0.18
C_2H_5	0.17	0.08	C_2H_5S	0.23	0.90
CH_3O	0.04	0.05	CH_3NH	0.05	0.40
C_2H_5O	0.33	0.30	C_2H_5NH	0.66	0.48

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

Furthermore, the introduction of any substituents on the benzene ring, the hetero-ring, or the exocyclic ester group brings down the insecticidal activity[25,34]. (Table - 4) Thus Salithion, the simplest phosphorothionate, was the most promising compound as insecticide amongst all the series of Saligenin cyclic phosphorus esters.

Table - 4

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



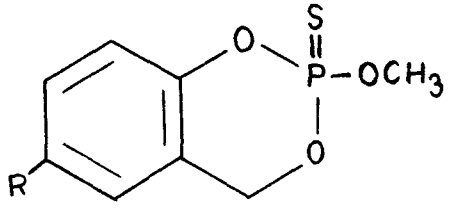
R	X		R	X	
	S	O		S	O
H	0.05 (Salithion)	0.035 (Salioxon)	6-Cl	1.75	0.09
4-CH_3	-	3.35	8-Cl	0.13	0.23
6-CH_3	2.00	0.10	β-CH_3	0.30	0.33
7-CH_3	0.23	0.43	β-CH_3OCH_2	3.55	0.99
8-CH_3	1.30	2.0	β-Cl	-	2.07

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

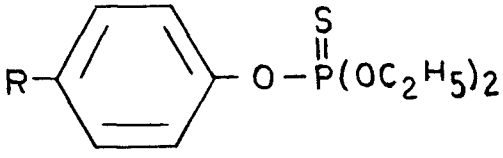
The reactivity of the cyclic phosphate ester of Saligenin is surprisingly greater than that expected from the acidity consideration of Saligenin, though the hetero-ring was not much strained and the endocyclic O-P-O angle of Salithion (104°) is in the range of angle of acylic phosphate esters ($102\text{-}108^{\circ}$)[4]. Many five and six-membered cyclic phosphorus esters have been prepared from 1,2-and 1,3-alkanediols and examined for antiesterase and insecticidal activities by Fukuto[35] and Edmundson[36]. These cyclic esters showed high reactivity but exhibited only poor anticholinesterase and insecticidal activities. Cyclic phosphorothionates of catechol inhibit plasma cholinesterase but showed almost no insecticidal activity[37]. Therefore, the high activity of Saligenin cyclic

Table - 5

Effect of p-substitution on insecticidal activity of Salithion (VII) and Parathion (VIII) series.



VII



VIII

R	δ[a]	Relative insecticidal activity[b]	
		VII	VIII
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.

phosphorus esters may be attributed to the special hetero-ring involving an enol and a benzyl ester linkage[17].

6. Activity as systemic insecticides:

Some known systemic insecticides, such as Schradan (Octamethyl Pyrophosphoramide) and Mipafox (N,N-diisopropyl phorodiamidic fluoride), have phosphoramide linkage. On analysis

it seemed probable that Saligenin cyclic phosphorus esters can also be endowed with systemic insecticidal activity by the introduction of an alkyl amino group on the phosphorus atom. Actually, Saligenin cyclic N-methyl phosphoramidate and phosphoramidothionate (2-methylamido-4H-1,3,2-benzodioxophosphorin-2-oxide and sulphide) revealed a considerable systemic insecticidal activity against rice stem-borer and green rice leafhoppers on rice plants[26].

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

7. Synergistic activity

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

At least two esterases having the ability to hydrolyse Malathion or its phenoxy carbonyl homologue were detected in the homogenate of the resistant strain G. One of these was

more potent and specific esterase for hydrolysis of Malathion. Both the esterases were completely inhibited by Saligenin cyclic phenyl phosphate[40]. For the resistant strains of red citrus mites, Panonychys citri (Mc Gregor), Saligenin cyclic phenylphosphonate displayed a high synergistic action with Malathion, by inhibiting the degradation of carboxylic ester linkage in Malathion Molecule[41]. 7-Methyl-2-phenyl-4H-1,3, 2-benzodioxaphosphorin-2-oxide is the most active synergist against resistant houseflies and green rice leaf-hoppers.

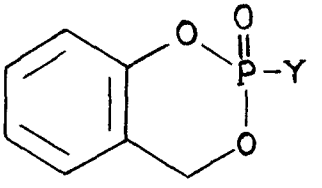
8. Antiesterase Activity:

Reference was already made that the specificity in biological activities of Saligenin cyclic phosphorus esters were remarkably influenced by the steric characteristics of the exocylic substituent on the phosphorus atom. This was evident when one compared their specificities in enzyme inhibition[30]. Saligenin cyclic phosphorus esters reacted with nucleophilic agents, including esterases, to phosphorylate by opening of the cyclic ester structure at P-O-C (aryl) bond (Fig. 6)[6,29,42].

The chemical and biological activities of three representative Saligenin cyclic esters, methyl phosphate, phenyl phosphate and phenyl phosphonate are compared in Table - 6. The insecticidal methyl phosphate was very active as an inhibitor of cholinesterase. However, the highly neurotoxic aryl phosphate was a poor inhibitor of cholinesterase but a very specific inhibitor of aliesterase. The less neurotoxic aryl phosphonate occupied an intermediate position.

Table - 6

Effect on substituents on chemical and biological activities.



Y	K_{hyd} x10^3 min^{-1}	I_{50} ChE x 10^{-8} M (C)	I_{50} AliE x 10^{-8} M (A)	C/A	LD_{50}^{*}	Synergism**	Ataxia***
OCH_3	1.4	7.6	8.4	0.9	0.04	0.6	n.a.
OC_6H_5	6.3	155	1.4	119	>10(3)	2.3	2
C_6H_5	12.8	89	3.2	27.8	>10(0)	2.5	200

* μg/housefly;

** Cotoxicity Coefficient;

*** Minimum ataxic dose in hens (mg/kg);

n.a. = No ataxia.

9. Nematocidal Activity:

A number of Saligenin cyclic phosphorus esters were effective to kill nematodes[29]. N-Methylphosphoramidate is most active but N,N-dimethylphosphoramidate was inactive against the non-parasitic soil nematode Rhabditis suspended in water[26]. Owing to instability in water, the cyclic phosphates and phosphonates were practically inactive but their thionoanalogues were considerably active against Rhabditis[24].

Some Saligenin cyclic aryl phosphonothionates were more effective against the rice white tip nematode (Aphelenchoides besseyichristie) than the cyclic N-methyl phosphoramidate, though the formers were poor in insecticidal activity[43]. These arylphosphonothionates also showed a high activity against filaria in cotton rats (Litomosoides carinii). It was interesting to note that these arylphosphonothionates were poor insecticides, whereas they were more potent to other critical target of nematodes. So they differ in nature from the insect cholinesterase[17].

10. Fungicidal Activity:

Recently some phosphorothiolate esters, particularly having S-benzyl ester-linkage, have been developed as fungicides. S-Benzyl-O, O-diethyl phosphorothiolate (Kitazin) is a typical fungicide now used in practice for control of rice blast disease. S-Alkyl Saligenin cyclic phosphorothiolate esters, which had no S-benzyl ester-linkage but O-benzyl and S-alkyl ester linkages, were examined for fungicidal activity[44,27]. They were found active not only as insecticides but also as fungicides. Some of them are effective to protect rice plants from rice-blast disease caused by the infection of Pyricularia oryzae. Ethyl and n-butyl esters were most promising as fungicides

The S-alkyl cyclic phosphorothiolates inhibit not only serine enzymes including cholinesterase but also SH-enzymes such as Papain also alcohol dehydrogenase[45,46]. The activity to inhibit SH-enzymes appeared to relate to fungicidal activity.

The cyclic phosphorothiolates (a) were highly reactive and rapidly hydrolyzed by ring opening to form the partial hydrolyzates, S-alkyl O-Salicyl hydrogen phosphorothiolates (b) which reacted readily with nucleophiles like mercaptans to alkylate them.

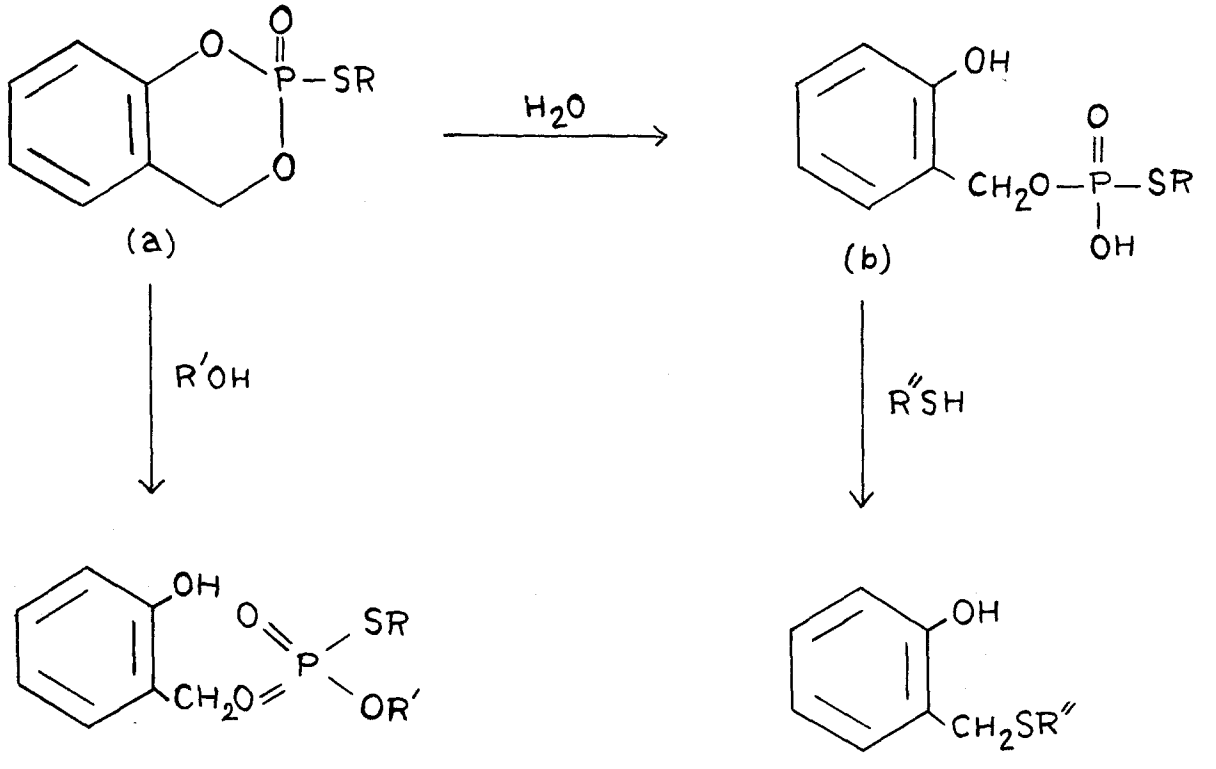
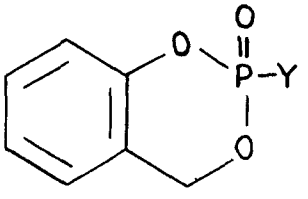
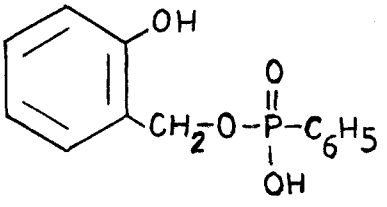


Fig. 7 Phosphorylation and alkylation reactions by Saligenin cyclic phosphorothiolates. (R'OH = alcohol and serine enzyme; R"SH = mercaptans and SH-enzymes).

Table - 7

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

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

* S-substituted cysteine was produced (+) or not (-) at pH 7.6, 28°C for 30 min.

** No inhibition occurred at 5×10^{-4}M.

*** Cyclohexylammonium salt was used.

It was the open-ringed o-hydroxy benzyl ester which actually inhibited the alcohol dehydrogenase instead of saligenin cyclic hydrogen phosphate (Table - 7)[8]. The hydroxy

group in the ortho position may promote the alkylating property of benzyl ester, giving a benzyl carbonium ion[45].

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

11. Conclusion:

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

R E F E R E N C E S

1. Smith, M.I., Elvove, E. and Frazier, W.H. : U.S. Pub. Health Rep. 45, 2509-2524, (1930).
2. Smith, H.V. and Spalding, J.M.K. : Lancet 2, 1019-1021, (1959).
3. Aldridge, W.N. and Barnes, J.M. : Biochem. Pharmacol 6, 177-188, (1961).
4. Eto, M. : Organophosphorus Pesticides : Organic and Biological Chemistry, CRC Press, Cleveland, (1974).
5. Casida, J.E., Baron, R.L., Eto, M. and Engel, J.L. : Biochem. Pharmacol, 12, 73-83, (1963).
6. Eto, M., Casida, J.E. and Eto, T. : Ibid 11, 337-352, (1962)
7. Eto, M. : Resid. Rev. 25, 187-200, (1969).
8. Eto, M. : Pesticide Chemistry Vol. 1, 'Insecticides'. Ed. by Tahori, A.S., Gordon and Breach Science Pub., London, 311-323, (1972).
9. Eto, M., Oshima, Y. and Casida, J.E. : Biochem. Pharmacol, 16, 295-308, (1967).
10. Taylor, J.D. and Butter, H.S. : Toxicol. Appl. Pharmacol. 11, 529-537, (1967).
11. Eto, M., Matsuo, S. and Oshima, Y. : Agr. Biol. Chem. 27, 870-875, (1963).
12. Eto, M., Kinoshita, Y., Kato, T. and Oshima, Y. : Nature 200, 171-172, (1963).
13. Eto, M. and Oshima, Y. : Agr. Biol. Chem. 26, 452-459, (1962
14. Oshima, Y. and Eto, M. : U.S. Patent 3, 478, 133, (1969).
15. Eto, M., Kinoshita, Y., Kato, T. and Oshima, Y. : Agr. Biol. Chem. 27, 789-794, (1963).
16. Kobayashi, K., Eto, M., Hirai, S. and Oshima, Y. : J. Agr. Chem. Soc. Japan 40, 315-317, (1966).
17. Eto, M. : Review of Plant Protection Research, Tokyo, Japan, Vol. 9, 1-20, (1976)
18. Sumitomo Chemical Co. : British Patent 1, 228, 121 : Chem. Abst. 75, 34493, (1971)

19. Eto, M., and Miyamoto, J : Analytical Method for Pesticides and Plant Growth Regulators, Vol. VII, Ed. by Zweig, G., Academic Press, New York, p. 431-440, (1973)

20. Eto, M., Nagata, R. and Oshima, Y. : J. Agr. Chem. Soc. Japan, 39, 311-316, (1965).

21. Sasaki, M., Ohkawa, H. and Eto, M. : J. Fac. Agr., Kyushu Univ. 17, 173-180, (1973).

22. Eto, M., Sasaki, M., Iio, M., Eto, M.Y. and Ohkawa, H. : Tetrahedron Letters 45, 4263-4266, (1971).

23. Sasaki, M. and Mukai, K. : Japan Kokai 74, 26, 290; Chem. Abst. 81, 388, (1974).

24. Eto, M., Kishimoto, K., Matsumura, K., Oshita, N. and Oshima, Y. : 30, 181-185, (1966).

25. Eto, M., Kobayashi, K., Sasamoto, T., Cheng, H.M., Aikawa, T., Kume, T. and Oshima, Y. : Botyu-Kagaku, 33, 73-77, (1968).

26. Eto, M., Kobayashi, K., Kato, T., Kojima, K. and Oshima, Y. : Agr. Biol. Chem. 29, 243-248, (1965).

27. Kobayashi, K., Eto, M., Oshima, Y., Hirano, T., Hosoi, T. and Wakamori, S. : Botyu-Kagaku, 34, 165-169, (1969).

28. Eto, M., Oshima, Y., Kitakato, S., Tanaka, F. and Kojima, K. : Botyu-Kagaku, 31, 33-38, (1966).

29. Eto, M., Eto, T. and Oshima, Y. : Agr. Biol. Chem., 26, 630-634, (1962).

30. Eto, M., Hanada, K., Namazu, Y. and Oshima, Y. : Agr. Biol. Chem., 27, 723-727, (1963).

31. Johnson, M.K. : Biochem., J. 120, 523-531, (1970).

32. Johnson, M.K. : Biochem. Pharmacol. 24, 797-805, (1975).

33. Aldridge, W.N. and Johnson, M.K. : Bull. World Health Org. 44, 259-263, (1971).

34. Kobayashi, K., Hirano, T., Wakamori, S., Eto, M. and Oshima, Y. : Botyu-Kagaku 34, 66-69, (1969).

35. Fukuto, T.R. and Metcalf, R.L. : J. Med. Chem., 8, 759, (1965).

36. Edmundson, R.S. and Lambie, A.J. : J. Chem. Soc. (C) 1997, 2001, (1966).

37. Tichy, V., Rattay, V., Janok, J. and Valentinova, I. : Chem. Zuesti 11, 398, (1957).

38. Ohkawa, H., Eto, M. and Oshima, Y. : Jap. J. Appl. Ent. Zool. 14, 191-194, (1970).

39. Takahashi, Y., Saito, T., Iyatomi, K. and Eto, M. : Botyu-Kagaku 37, 13-23, (1972).

40. Ohkawa, H., Eto, M., Oshima, Y., Taneka, F. and Umeda, K. : Botyu-Kagaku 33, 139-145, (1968).

41. Takahashi, Y., Saito, T., Iyatomi, K. and Eto, M. : Botyu-Kagaku 38, 13-21, (1973).

42. Eto, M. and Oshima, Y. : Agr. Bio. Chem., 26, 834-841, (1962).

43. Mihara, K. and Eto, M. : Sci. Bull. Fac. Agr. Kyushu Univ. 30, 105-108, (1975).

44. Eto, M., Ohkawa, H., Kobayashi, K. and Hosoi, T. : Agr. Biol. Chem., 32, 1056-1058, (1968).

45. Ohkawa, H. and Eto, M. : Agr. Biol. Chem., 33, 443-451, (1969).

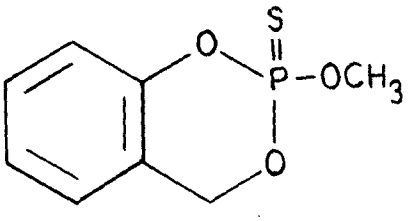
46. Eto, M. and Ohkawa, H. : Biochemical Toxicology of Insecticides. Ed. by O'Brien, R.D. and Yamamoto, I., Academic Press, New York, p. 93-104, (1970).

PART - II

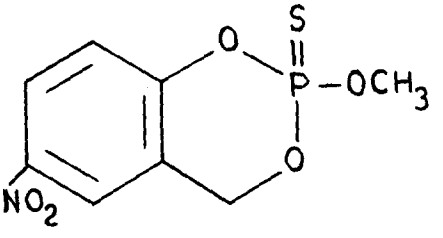
AIM AND OBJECTIVES OF THE PRESENT INVESTIGATION

As stated previously (Ref. Part - I) the discovery of Saligenin cyclic phosphate as a biologically active metabolite of tri-o-cresyl phosphate (TOCP)[1,2,3] led to the synthesis of many related compounds and to the study of their chemical and biological properties[4,5]. Analogous cyclic phosphorus esters have been synthesised to study their chemical properties[6,7] and biological activities[8,9]. Among the saligenin cyclic phosphorus esters, Salithion (2-methoxy-4H-benzo-1,3,2-dioxaphosphorin-2-sulphide) was discovered in 1963 in the laboratory of Pesticide Chemistry, Kynshu University, Japan and commercialized in 1968 by Sumitomo Chemical Company as a practical insecticide[1,5]. Compared to the high neurotoxicity of the TOCOP metabolite, Salithion causes no such toxicity. Introduction of any type of substituent at any position of the benzene ring of Salithion decreases the insecticidal activity[10]. It has been reported[10] that 2-methoxy-6-nitro-4H-benzo-1,3,2-dioxaphosphorin-2-sulphide (BD-8) is obtained as a paste in the reaction of 2-hydroxy-5-nitro benzyl alcohol with methyl phosphorodichloridothionate after purification through silicic acid column chromatography, and this methoxy compound has about sixty times less insecticidal

activity compared to Salithion[10]. In this laboratory it was observed[11] that the methoxy compound (BD-8) is a solid (m.p. 84°C) and has about 1.5-2.0 times greater insecticidal activity against Cockroach, Periplaneta americana (Linn.), and comparable activity against Grasshoppers, Oxya nitidula compared to Salithion; it also decomposes more easily than Salithion leaving less residues in the environment[11].

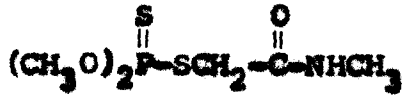


Salithion

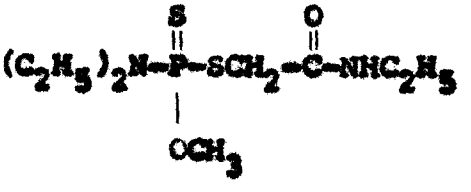


BD-8

Moreover, introduction of an amine group in place of an alkyl ester group often affords organophosphorus ester with fungicidal activity[12]. For example, the insecticide dimethoate [dimethyl S-(N-methylcarbamoylmethyl) phosphorothiolothionate] has no fungicidal activity, its dialkyl phosphoramidothiolothionate analogs, such as compound-I, shows some fungicidal as well as acaricidal activity[13].



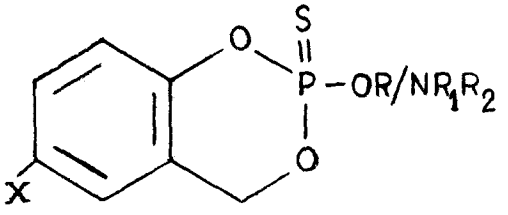
(DIMETHOATE)



(COMPOUND - I)

Several other examples in literature clearly show that some phosphoramidothionates, phosphoramidothiolothionates, phosphoramides or phosphonamides in which the phosphorus atom is attached directly to the nitrogen atom of an amino or a heterocyclic compound such as phthalimide, imidazole or triazole, have very good fungicidal activity[1,12,14,15].

The above observations prompted us to undertake a systematic work on some 2-alkylamido/alkoxy-6-nitro/bromo/chloro-4H-benzo-1,3,2-dioxaphosphorin-2-sulphides having general structure (A).



where $R/R_1/R_2$ = Alkyl group

X = Cl, Br, NO_2

(A)

The alkoxy groups are isopropoxy, methoxy and ethoxy groups, the alkylamido groups are Pyrrolidino, Piperidino, 2-ethyl-piperidino and hexamethyleneimino.

The work embodied in this dissertation is related to the investigation of the above mentioned compounds with reference to their chemical, pesticidal and toxicological properties besides structure elucidation by spectroscopic methods.

Actual work:

(i) Some 2-alkoxy/alkyl amido-6-chloro/bromo/nitro-4H-1,3,2-benzodioxaphosphorin-2-sulphide have been synthesized; the structure of these compounds have been determined by taking UV, IR, NMR and Mass spectra.

(ii) Insecticidal activities against Cockroach (Periplaneta americana), Blow-fly (Chrysomaya megacephala), Grasshopper (Oxya nitidula) and Aphids (Lipaphis erysimi) have been reported.

(iii) Acute oral toxicity and delayed neurotoxicity tests on white albino rats and hens were carried out.

(iv) Inhibition of acetylcholinesterase activity against blow-fly head homogenate and goat whole blood have been reported.

(v) Antifungal activities against Helminthosporium oryzae and Pyricularia oryzae have been studied.

(vi) Phytotoxic properties on the germination of wheat seed (Triticum sp.) have been studied.

(vii) Cytotoxicity tests on plant (onion) and animal (mouse) were also studied.

(viii) Chemical hydrolysis of these compounds have been studied in alkaline solution (pH 11.85, 7.7).

R E F E R E N C E S

1. Eto, M. : Organophosphorus Pesticides; Organic and Biological Chemistry, CRC Press, Cleveland, Ohio (1974).

2. Casida, J.E., Eto, M. and Baron, R.L. : Nature, 191, 1396(1961).

3. Eto, M., Casida, J.E. and Eto, T. : Biochem. Pharmacol. 11, 337 (1962).

4. Eto, M. : Residue Reviews, 25, 187 (1969).

5. Eto, M. : Review of Plant Protection Research, 9, 1 (1976).

6. Eto, M., Oshima, Y. : Agric. Biol. Chem., 26, 452 (1962).

7. Eto, M. and Oshima, Y. : Agric. Biol. Chem. 26, 834 (1962).

8. Eto, M., Eto, T. and Oshima, Y. : Agric. Biol. Chem. 26, 630 (1962).

9. Casida, J.E., Baron, R.L., Eto, M. and Engel, J.L. : Bio-Chem. Pharmacol., 12, 73 (1963).

10. Eto, M., Kobyashi, K., Sasamoto, T., Cheng, H.M., Ackawa, T. Kume and Oshima, Y. : Botyukagaku, 33, 73 (1968).

11. (a) Das, B.K. : Pesticides, 15, 3 (1981).
    (b) Das, B.K. : D.Sc. Thesis, Calcutta University (1981).

12. Grapov, A.P. and Melnikov, N.N. : Russ. Chem. Rev., 42(9), 776 (1973).

13. Mandelbaum, Ya. A., Saifer, R.S., Melnikov, N.N. and Fedoseenko, L.G. : Khim-Sel'sk Kohz., 6, 107 (1968).

14. Fest, C. and Schmidt. : The Chemistry of Organophosphorus Pesticides, Springer-Verlage, p. 148-151 (1973).

15. Tolkimth, H. : Nature, 211 (5048), 522 (1966), Science 155, 85 (1967).

CHAPTER - 2

PART - I

MATERIALS AND METHODS

All melting points were taken in open capillary tubes in sulfuric acid bath and are uncorrected.

1. Purification of solvents and other chemicals:

Organic solvents and chemicals used during the work were of standard commercial grade of high quality (EM/BDH/SM/ Fluka/Aldrich and Sigma quality). Organic solvents and other chemicals were purified and dried according to Vogel[1]. Silica gel (60-120 mesh) was used for column chromatography.

2. Spectroscopic methods:

Infra red spectra were scanned on Beckmann IR-20 spectrophotometer and Pye Unicum SP 3 300S in nujol mull and in liquid film. Ultraviolet-spectra were recorded on Beckmann DU-2 Spectrophotometer and Shimadzu UV-Visible Recording Spectrophotometer (UV-240) in ethanol. Mass spectra (EI positive) were recorded on a Jeol JMS-D 300 mass spectrometer at 70 eV. PMR spectra were taken on Varian EM 360-L, Varian CFT-20 and Jeol spectrophotometers. Chemical shifts in parts per million for ^{1}H NMR spectra were referenced to Me_4Si. Solvents used for NMR spectra were Chloroform-d and Acetone - d_6.

3. Preparation of Thiophosphoryl Chloride ($PSCl_3$):

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

4. Preparation of 5-Chloro-Saligenin (2-Hydroxy-5-Chloro-Benzyl alcohol).

2-hydroxy-5-chloro-benzyl alcohol as one of the starting materials for the synthesis of chloro-saligenin cyclic alkylamido phosphorothionates was prepared in the following manner.

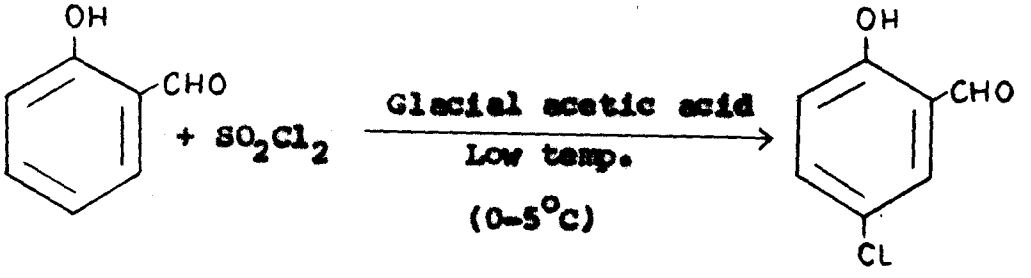
Preparation of the chloro alcohol was done in two stages.

(i) Prepration of 2-hydroxy-5-chlorobenzaldehyde, and

(ii) Reduction of the said aldehyde to alcohol.

4.(i) Chlorination of Salicylaldehyde:

Chlorination of salicylaldehyde was done by sulphuryl-chloride-acetic acid.

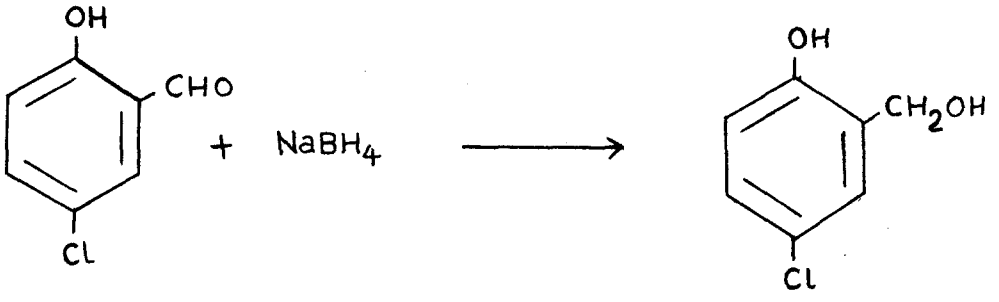


12.2g (0.1 mole) salicylaldehyde and 13.5g (0.1 mole) liquid sulphuryl chloride were dissolved in about five times glacial

acetic acid respectively in two separate conical flasks. The sulfuryl chloride solution was added slowly to salicylaldehyde solution with constant stirring to ensure thorough mixing. The temperature of the reaction mixture was kept at 0° to 5°C. After complete addition of sulfuryl chloride the reaction mixture was allowed to stand for half an hour at room temperature. The reaction mixture was poured in cold water (ordinary). The precipitate was filtered with suction on a Buchner funnel and was washed thoroughly with cold water and was pressed as dry as possible with a wide glass stopper and then dried. The product thus obtained was recrystallized from hot methanol. The yield of pure 2-hydroxy-5-chlorobenzaldehyde (colourless crystals, m.p. 100°C) was 12.8g (85% yield).

4.(ii) Reduction of 2-hydroxy-5-chlorobenzaldehyde:

The reduction was done by sodium borohydride according to Vogel[1].



In a 500 ml three-necked flask, equipped with a mechanical stirrer, a thermometer and a dropping funnel was placed, a solutic of 20.1g (0.1 mole) 2-hydroxy-5-chlorobenzaldehyde in 100 ml methanol and, whilst stirring, a solution of sodium bromohydride (1.40g, 0.03 mole sodium bromohydride in 2 ml of 2M-sodium hydroxide diluted with 18 ml of water) was added at a rate of 0.5 ml per minute with occasional cooling to keep the reaction mixture at 18-25°C. The reaction mixture was stirred for an additional period of 30 minutes.

The methanol was removed by distillation on a steam bath. The residue was diluted with 100 ml water and then acidified with 50 ml dilute sulphuric acid. The mixture was extracted with ether the upper layer of the extract was washed with a little anhydrous magnesium sulphate. Ether was removed by flash distillation. The product thus obtained was recrystallised from hot chloroform and dried in vacuum. The yield of pure 2-hydroxy-5-chlorobenzyl alcohol (colourless crystals, m.p. 89°C) was 83%.

UV (Fig. 1): λ_{max}^{EtOH} = 285 nm (ϵ = 3032)

IR (Fig. 2) :

3420 and 3140 cm^{-1}	: OH vibration;
1425 cm^{-1}	: OH deformation str. vibration;
1610 cm^{-1}	: C = C str.;
1480 cm^{-1}	: $-CH_2$ - scissoring;
1080 cm^{-1}	: C-O str. of primary alcohol;
1210 cm^{-1}	: C-O str. of phenol;
895 cm^{-1}	: lone H-atom wagging of the phenyl ring;
810 cm^{-1}	: bending of the 2H adjacent of the ring.

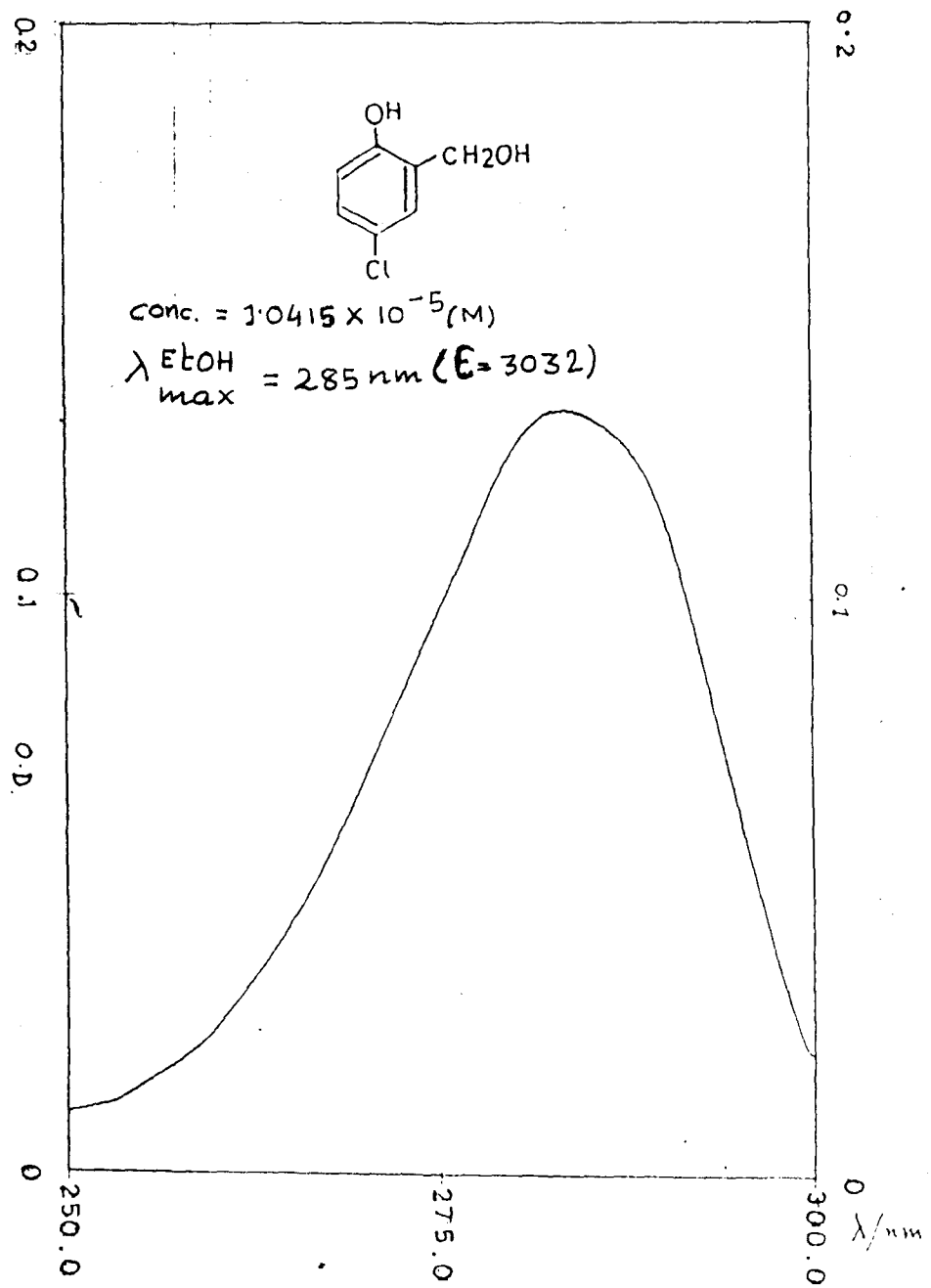


Fig. 1 UV spectrum of 5-chloro saligenin in ethanol.

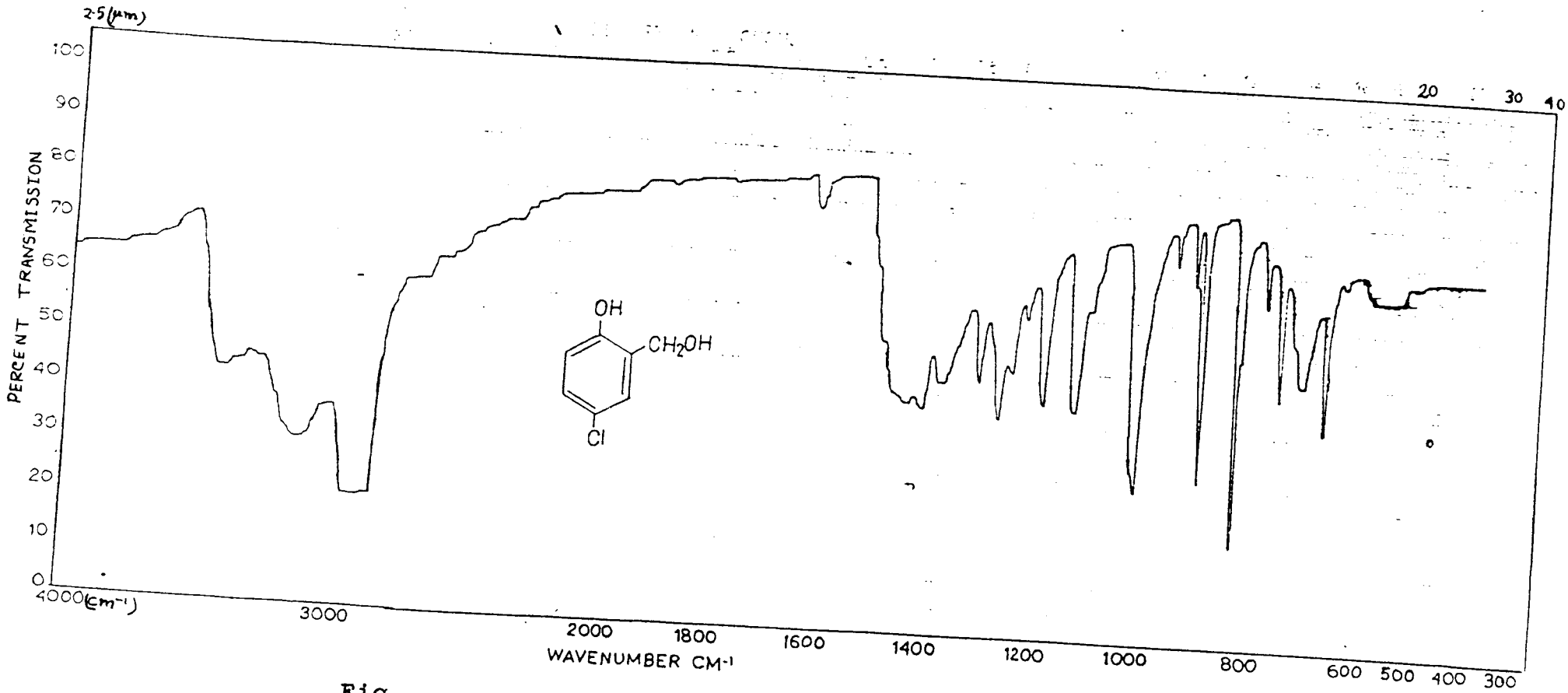


Fig. IR spectrum of 5-chloro saligenin.

5. Preparation of 5-Bromo-saligenin (2-Hydroxy-5-Bromobenzyl alcohol:

2-hydroxy-5-Bromobenzyl alcohol as one of the starting materials for the synthesis of Bromosaligenin cyclic alkylamido phosphorothionate was prepared in following manner.

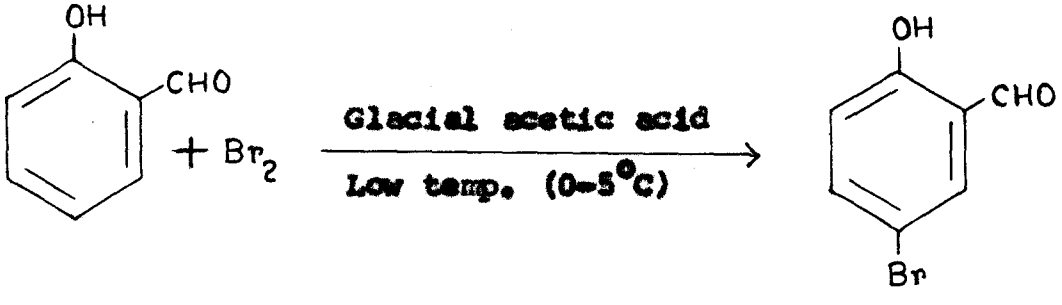
Preparation of the Bromoalcohol was done in two stages.

(i) Preparation of 2-hydroxy-5-Bromobenzaldehyde, and

(ii) Reduction of the said aldehyde to alcohol.

5.(i) Bromination of salicylaldehye:

Bromination of salicylaldehyde was done by bromine acetic acid method according to Vogel[1].

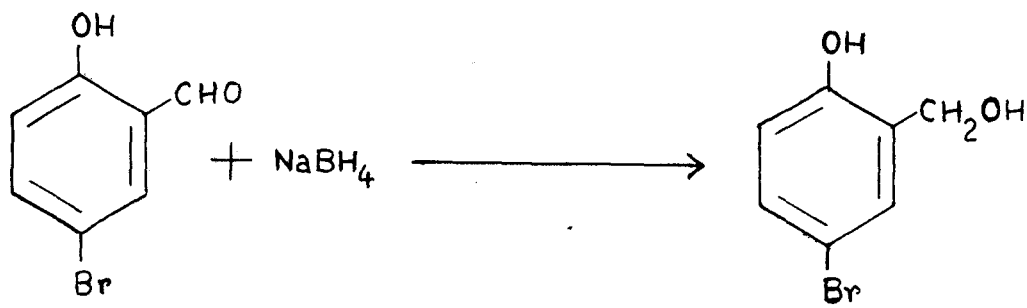


12.2g (0.1 mole) salicylaldehyde and 17g (0.1 mole) liquid bromine were dissolved in 45 ml and 25 ml glacial acetic acid respectively in two separate conical flasks. The bromine solution was added slowly to salicylaldehyde solution with constant shaking to ensure thorough mixing. The temperature of the reaction mixture was kept at 0° to 50°C. After complete addition of bromine, the reaction mixture was allowed to stand at room temperature for an additional period of 30 minutes with occasional shaking, while fine crystals were separated; the whole reaction

mixture was then poured in to 400 ml cold water and more were obtained. The crystalline mass was filtered ; washed repeatedly with cold water to remove excess bromine and then dried. The product thus obtained was recrystallized from hot methanol. The yield of pure 2-hydroxy-5-bromobenzaldehyde (colourless crystals, m.p. 103°C) was 16.1g (80% yield).

5.(ii) <u>Reduction of 2-hydroxy-5-bromo-benzaldehyde</u>:

The reduction of 2-hydroxy-5-bromobenzaldehyde to alcohol was done according to the method described in 4 (ii).



The yield of pure 2-hydroxy-5-bromo-benzyl alcohol (colourless crystals, m.p. 109°C) was 85%.

UV (Fig. 3): λ_{max}^{EtOH} 284 nm (ϵ = 2080)

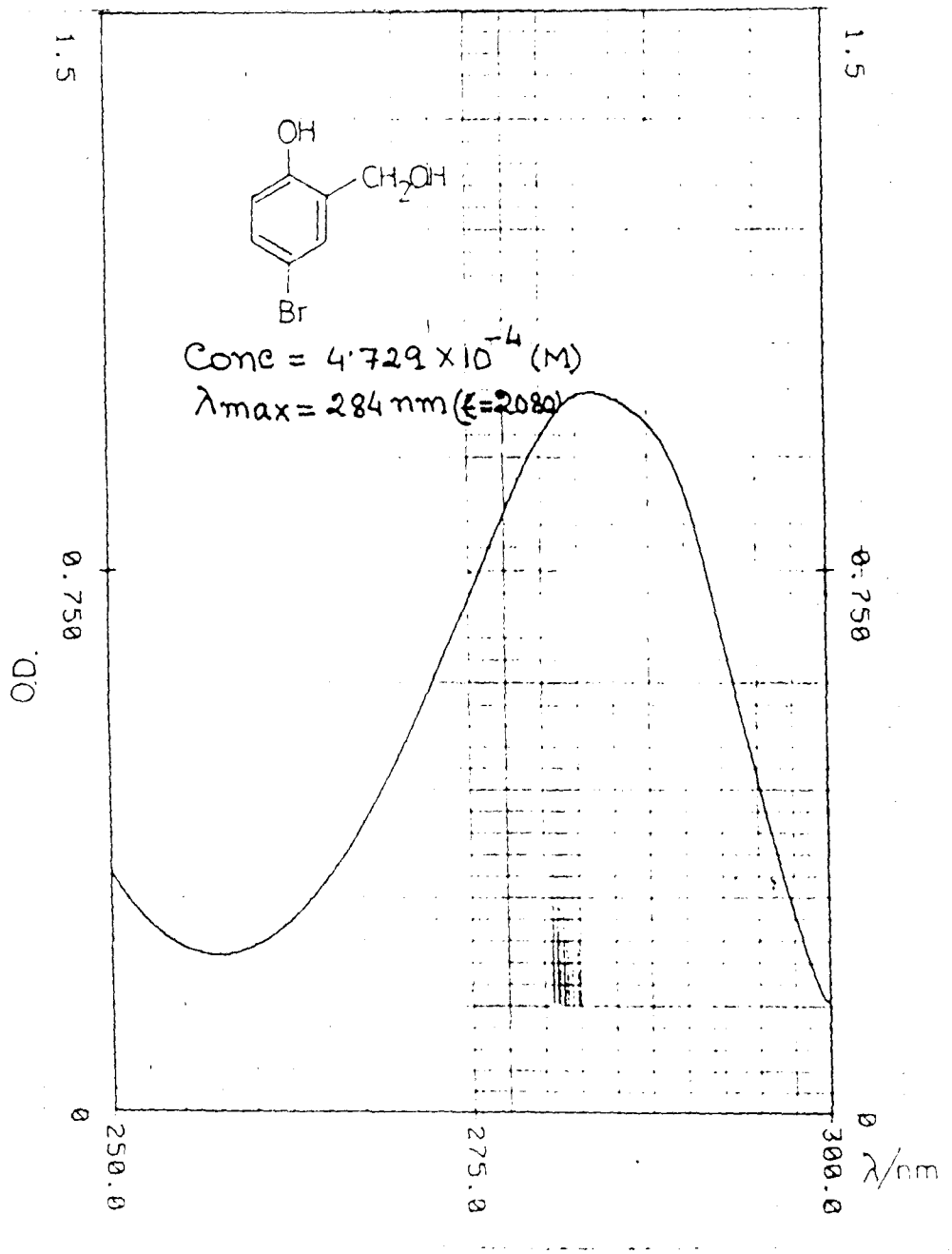


Fig. 3 UV spectrum of 5-bromo saligenin in ethanol.

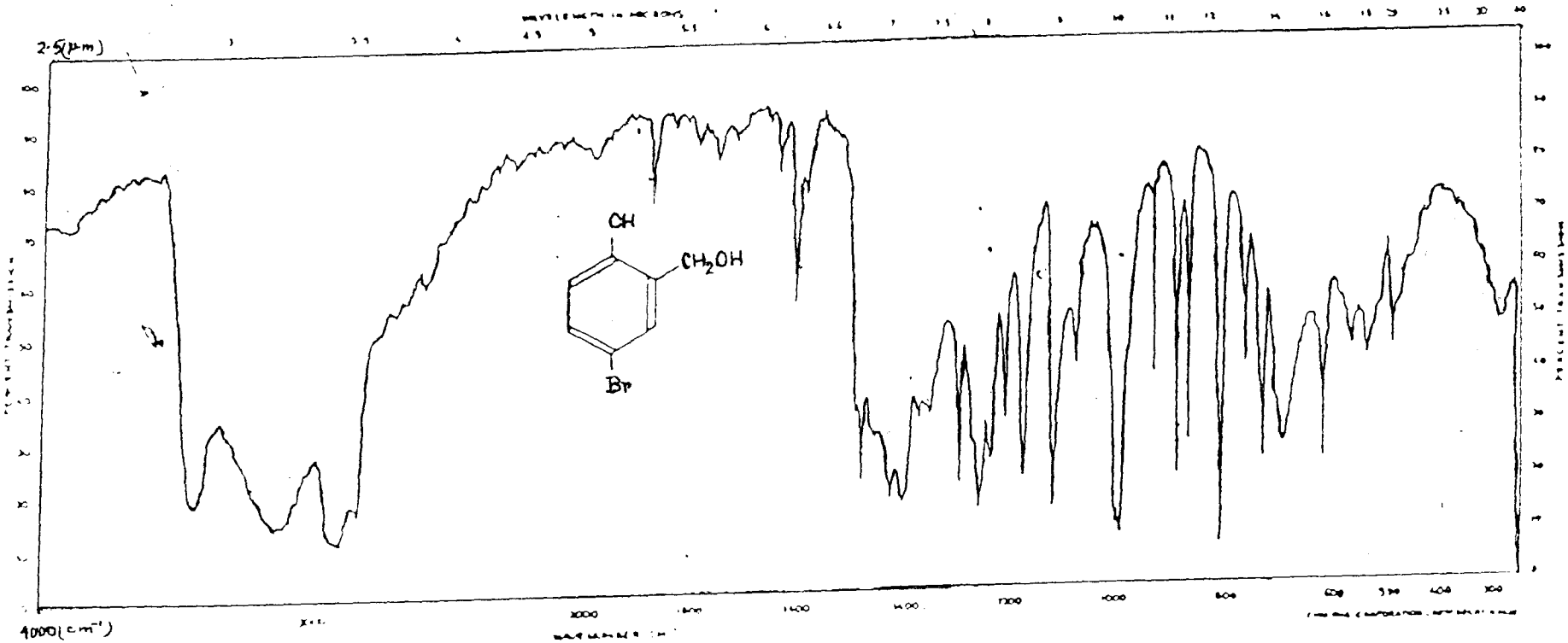


Fig.4 : IR spectrum of 5-bromo saligenin.

IR (Fig.-4):

3420 and 3140 cm^{-1}	: OH vibration;
1430 cm^{-1}	: CH deformation str. vibration;
1610 and 1490 cm^{-1}	: C = C str. ;
1480 cm^{-1}	: $-CH_2$ - scissoring;
1080 cm^{-1}	: C-O str. of primary alcohol;
1210 cm^{-1}	: C-O str. of phenol;
890 cm^{-1}	: long H-atom wagging of the phenyl ring;
810 cm^{-1}	: banding of 2H adjacent of the ring;

6. Preparation of 5-Nitro-saligenin (2-Hydroxy-5-Nitro-benzyl alcohol).

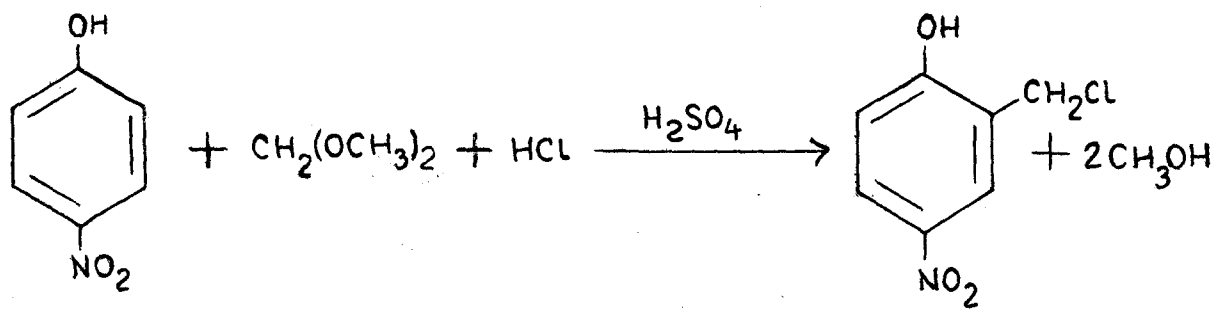
2-hydroxy-5-nitrobenzyl alcohol, one of the starting material for the synthesis of nitrosaligenin cyclic phosphoramidothionate was prepared in two steps.

(i) Preparation of 2-hydroxy-5-nitrobenzyl chloride, and

(ii) the hydrolysis of the said chloride.

6. (i) Preparation of 2-hydroxy-5-nitro-benzyl chloride

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



p-nitrophenol used here was purchased from the market (Reidel, m.p. 114°C) while the other ingredient, methylal was synthesised a fresh for every batch of preparation as follows.

To a mixture of 760 ml methanol, 400g anhydrous $CaCl_2$ and 10.2 ml conc. hydrochloric acid was taken in a 3 lit. round bottomed flask equipped with a reflux condenser was added 400g of 37-40% formaldehyde with constant cooling and stirring. The addition was done dropwise through a dropping funnel. It took about 2 hours to complete the addition of formaldehyde (highly exothermic reaction). Then the mixture in the flask was heated for a few minutes until the liquid began to boil vigorously. Methylal that came up quickly on the upper layer was collected by fractional distillation after an overnight standing. The 42-45°C fraction was collected and stored in a standard joint bottle in cold (freezer) before it was used.

The reaction between p-nitrophenol and methylal was carried out in a one litre, three necked round bottomed flask equipped with a short reflux condenser, a thermometer and a bent glass tube reaching sufficiently below into the flask were placed 50g (0.36 mole) p-nitrophenol, 650 ml conc. HCl, 5 ml conc. H_2SO_4 and 76g (1 mole) methylal. The reaction mixture was stirred while the temperature was maintained at 70° ± 2°C for 4-5 hours. During this time HCl gas was bubbled in to the reaction mixture through the bent glass tube. 2-hydroxy-5-nitro-benzyl chloride began to separate as a solid after about one and half hour. At the end of the reaction, the mixture was cooled in

an ice-bath for a period of 1-2 hours when more crystals separated. The solid material after filtration, was kept in air for several hours and then washed with benzene. About 40-45g of chloride was obtained (m.p. 129° - 130°C).

6.(ii) Hydrolysis of 2-hydroxy-5-nitrobenzyl chloride:

2-hydroxy-5-nitrobenzyl chloride in water was boiled gently to ensure complete hydrolysis. After boiling the hot solution was quickly filtered and then cooled. The light yellow crystals of 2-hydroxy-5-nitrobenzyl alcohol was obtained. The crystals (m.p. 122-126°C) were re-crystallized from hot water. The crystals were finally washed with cold dioxan: benzene (1:9) mixture, and dried in vacuum.

m.p. 128°C (literature m.p. 128-129°C)[3],

R_f 0.74 (in methanol).

UV (Fig. 5) : λ_{max}^{EtOH} = 230 nm (6728) and 322 nm (9190)

IR (Fig. 6):

3450 and 3100 cm^{-1}	: OH vibration;
1438 cm^{-1}	: OH deformation str. vibration;
1475 and 1335 cm^{-1}	: asym. and sym. str. of NO_2 group;
1610 and 1585 cm^{-1}	: C = C str. ;
1080 cm^{-1}	: C-C-O;
980, 960 and 925 cm^{-1}	: 1:2:4 trisubstituted benzene ring vibration;
900 cm^{-1}	: lone H-atom wagging of the phenyl ring;

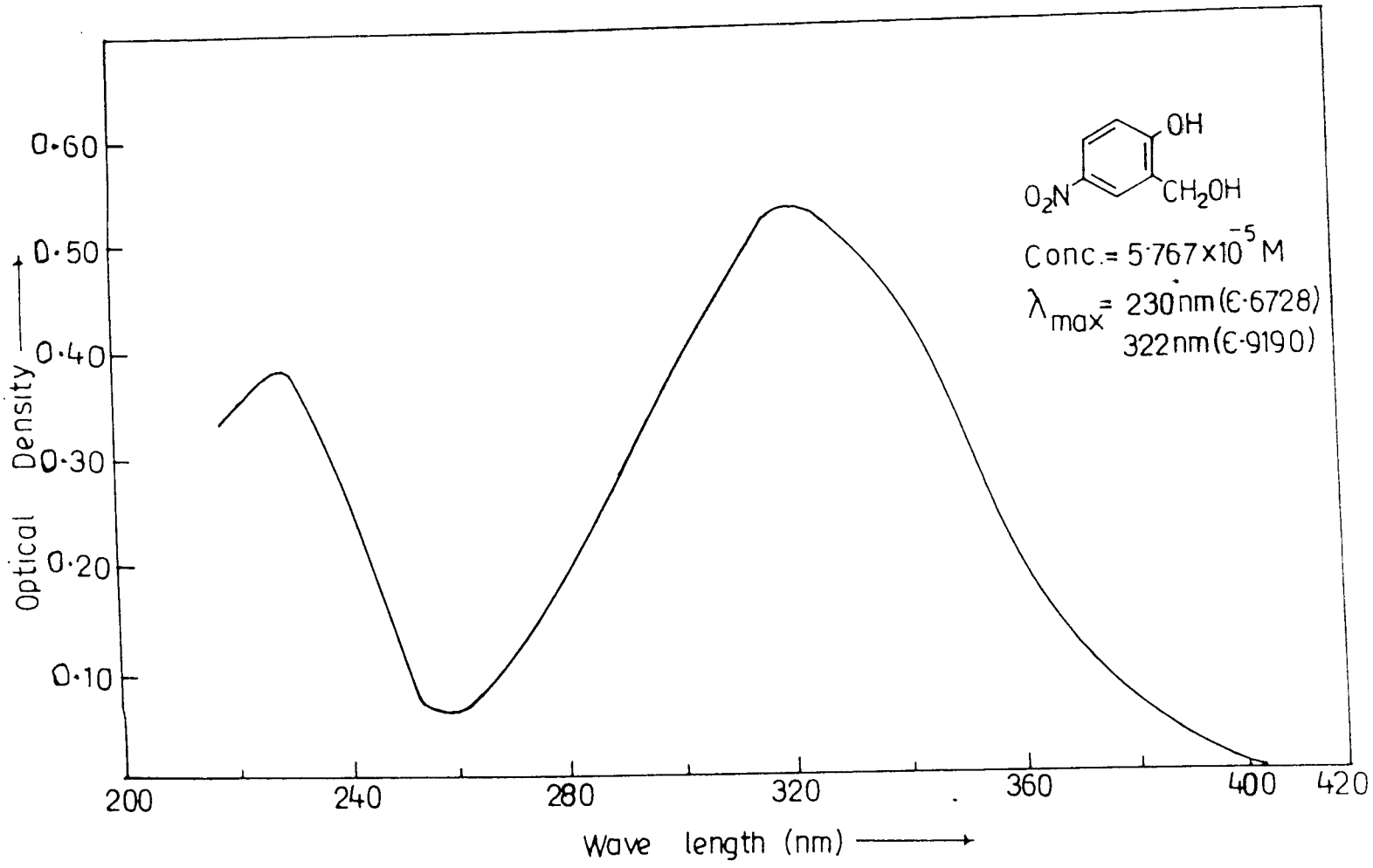


Fig. 5 UV spectrum of 5-nitro saligenin

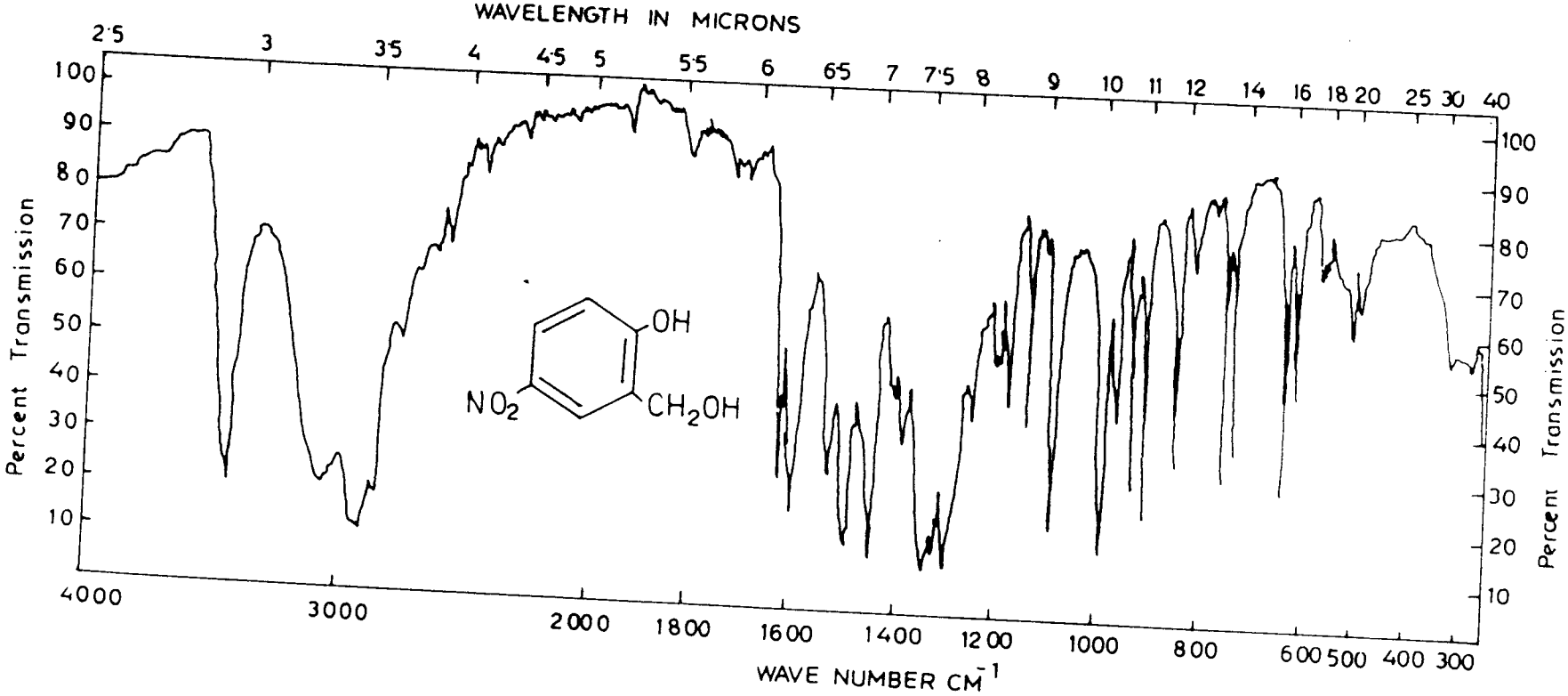
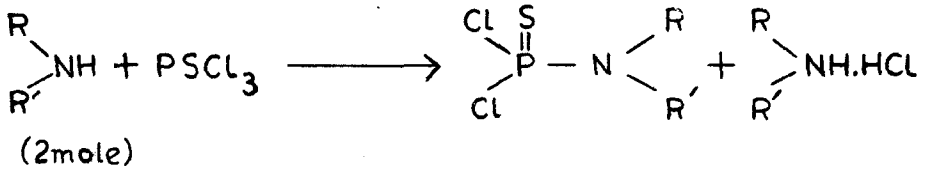


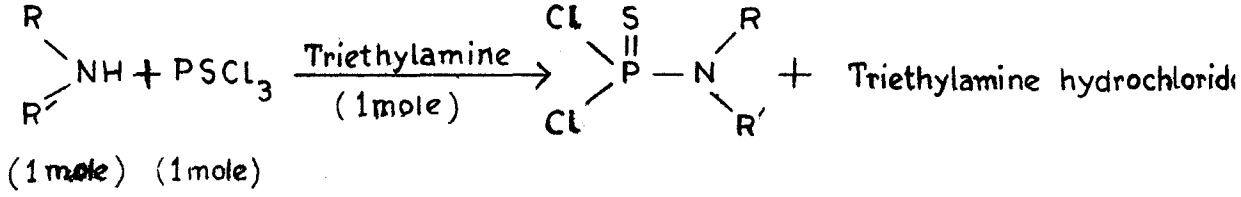
Fig. 6 IR spectrum of 5-nitro saligenin.

750 and 735 cm^{-1} : C-N-O bending;

810 cm^{-1} : 2H (adjacent) of the ring;

7. Preparation of Alkylamidophosphorodichloridothionates:





One mole $PSCl_3$ and two moles of amine (or, one mole amine and one mole triethylamine) were allowed to react at -5°C to +5°C in benzene/chloroform as solvent. The amine solution was added dropwise very slowly with constant vigrous stirring. After an additional stirring period, the solid particles (if present) were filtered off and the reaction mixture was washed repeatedly with benzene/chloroform. Excess amine was removed by washing the benzene/chloroform phase with 2% cold hydrochloric and then with cold saturated solution of sodium chloride. The benzene/chloroform

phase was then dried with anhydrous sodium sulphate and filtered, evaporation *in vivo* gave the desired alkylamidophosphorodichloridothionate. The different alkylamidophosphorodichloridothionates were prepared as follows:

7.(i) N,N-Diisobutylamidophosphorodichloridothionate:

A solution of diisobutylamine (12.9g; 0.1 mole) in 20 ml benzene was added dropwise to a stirred solution of thiophosphorylchloride (8.45g; 0.05 mole) in 50 ml benzene at -5°C to +5°C. The mixture was stirred at 5°C for 3 hours and then at room temperature for 16 hours. Diisobutylamine hydrochloride was filtered off, 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 dried with anhydrous sodium sulphate and filtered; evaporation *in vacuo* gave 10g. N,N-Diisobutylamidophosphorodichloridothionate. This compound has a camphor like odour.

7.(ii) N,N-Dibutylamidophosphorodichloridothionate:

Thiophosphorylchloride (8.45g, 0.05 mole) in 50 ml benzene was allowed to react with 6.5g (0.05 mole) of dibutylamine and 5g(0.05 mole) of triethylamine in 20 ml benzene, and the mixture was worked up as in 7(i). 5g liquid was obtained. This compound has a camphor like odour.

7.(iii) n-Butylamidophosphorodichloridothionate:

Thiophosphoryl chloride (0.05 mole, 8.45 gm) in 50 ml benzene was allowed to react with n-butylamine (0.1 mole, 7.3 gm) chloroform solution (25 ml). The temperature was maintained at

0° to $5^{\circ}C$ during addition. After addition of the amine, the reaction mixture was stirred for an additional period of 2 hours and then filtered. The filtrate was washed with cold 2% hydrochloric acid and then with cold saturated solution of sodium chloride. The benzene phase was then dried with anhydrous sodium sulphate and filtered. After evaporation *in vacuo*, a colourless liquid product was obtained.

7.(iv) n-Hexylamidophosphorodichloridothionate:

This compound was prepared by adding n-hexyl amine (10.1 gm 0.1 mole) in 20 ml benzene to thiophosphoryl chloride (8.45 gm, 0.05 mole) in 50 ml benzene; The reaction mixture was worked up as in 7(iii); 14 gm product was obtained as colourless viscous liquid.

7.(v) O-(β-methoxy)ethyl dichloridophosphorothionate:

This compound was prepared by mixing 17 gm $PSCl_3$ with 8.0 g pyridine in 75 ml benzene as solvent, followed by the addition of 7.6 gm (0.1 mole) methyl cellosolve; after working up the product obtained was 17.0 gm (80%).

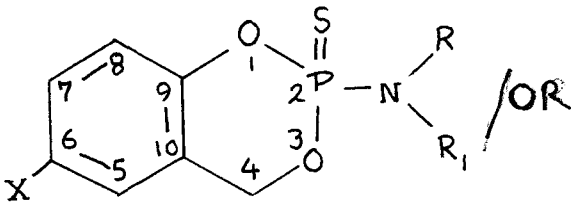
7.(vi) O-iso-propyl dichloridophosphorothionate:

The amount of the reactants and the solvent in the preparation were the same as that of the O-n-propyl isomer; the yield was 9.5 gm (50%).

7.(vii) O-phenyl dichloridophosphorothionate:

This compound was prepared by taking a mixture of 17.0 gm $PSCl_3$ and 8.0 gm pyridine in 50 ml benzene; 9.5 gm (0.1 mole) phenol was dissolved in 25m benzene and the resulting solution was added slowly to the above mixture. The reaction mixture, in this case, was stirred at room temperature ($27^{\circ}C$) for about 5-6 hours; after working up the product obtained was 16 gm (70%).

8. Preparation of some 2-alkylamido-6-chloro/bromo/nitro-4H-1, 3,2-benzodioxaphosphorin-2-sulphide:



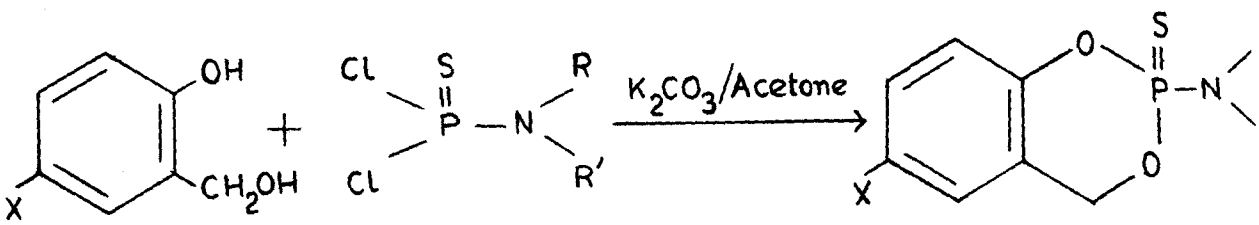
where X = Br, Cl, NO_2

and $-N\begin{matrix}R\\R_1\end{matrix}$ = secondary amino groups

R = alkyl groups

General Procedure

2-alkylamido-6-chloro/bromo/nitro-4H-1,3,2-benzodioxaphosphorin 2-sulphides were prepared by adding a solution of 2-hydroxy-5-chloro/bromo/nitro benzylalcohol (5-chloro/bromo/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. The temperature of the reaction mixture was strictly maintained below 5°C during the addition of potassium carbonate. The condensation was accomplished by stirring at the temperature 5-27°C for an additional time of 12-16 hours. The solid particles were filtered out of the reaction mixture and the solvent was removed uncer reduced pressure at room temperature. In some cases the crude product was directly recrystallized from methanol to give the pure compound; while in others an additional chloroform extraction was necessary prior to recrystallization. In the latter case, the crude product was extracted with chloroform and washed with 1% dil. HCl (ice-cooled) and with cold water; repeatedly. This was then dried with anhydrous sodium sulphate and the chloroform was subsequently removed under reduced pressure. The pure compound was then obtained by recrystallization from methanol.

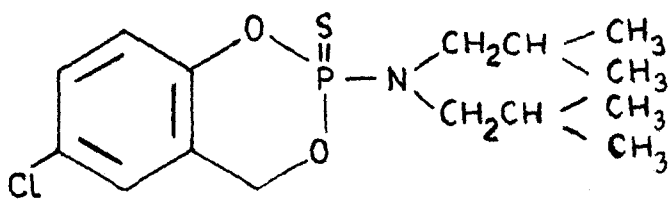


where , X = Br, Cl, NO_2

and, $-N\begin{matrix}R\\R'\end{matrix}$ = secondary amino groups.

The different phosphoramidothionates were prepared as follows:

8. (i) <u>2-N,N-Diisobutylamido-6-chloro-4H-1,3,2-benzodioxaphosphorin-2-sulphide (Cl-6)</u>:



This compound (Cl-6) was prepared by condensation of equimolar quantities of 1.59g (0.01 mole) of 2-hydroxy-5-chlorobenzyl alcohol and 2.50 ml (0.01 mole) of N,N-Diisobutyl-amidophosphorodichloridothionate in presence of 2.76g (0.02 mole) of anhydrous potassium carbonate in 50 ml acetone as solvent; potassium carbonate was added by instalments to the stirred solution at 0° to $5^{\circ}C$. After an additional stirring for 16 hours

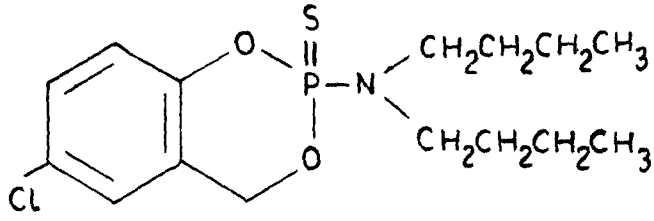
at the room temperature, the solids were filtered off, and the solvent was removed under reduced pressure. The crude product was washed with methanol saturated with n-heplane, and then the compound was recrystallised from hot methanol. 2.7g of compound was obtained as a white crystalline solid, m.p. 91°C, R_f 0.91 (benzene-acetone, 8:2), Yield, 78%; Mol. wt. 347.5; Molecular formula $C_{15}H_{23}O_2NPSCl$.

Analysis :

Found : C, 36.0%; H, 6.50%; N, 5.30%

Calculated for $C_{15}H_{23}O_2NPSCl$: C, 36.65%; H, 6.87%; N, 5.34%.

6.(ii) 2-N,N-Dibutylamido-chloro-4H-1,3,2-benzodioxaphosphorin-2-sulphide (CL-10):



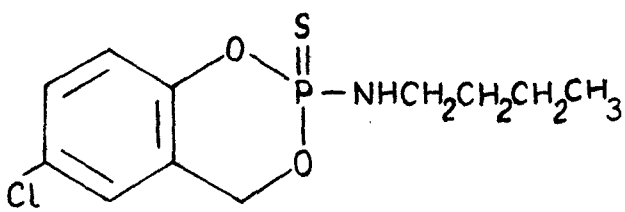
A solution of equimolar quantities of 1.59g(0.01 mole of 2-hydroxy-5-chlorobenzyl alcohol and 2.62g (0.01 mole) of N,N, dibutylamidophosphorodichloridothionate in 50 ml of dry acetone in presence of 2.76g (0.02 mole) of anhydrous potassium carbonate. The reaction and purification were carried out in same manner as that in 8(i). Removal of solvent in vacuo gave 2.6g of colourless liquid product CL-10.

Yield 75%; Mol. formula, $C_{15}H_{23}O_2NPSCl$; Mol. wt. 347.5; R_f 0.86 (benzene -acetone, 8:2).

Analysis:

Found	: C, 51.57%; H, 6.50%; N, 4.10%
Calculated for $C_{15}H_{23}O_2NPSCl$	: C, 51.70%; H, 6.61%; N, 4.02%

8. (iii) 2-N-Butylamido-6-chloro-4H-1,3,2-benzodioxaphosphorin-2-sulphide (Cl-24):

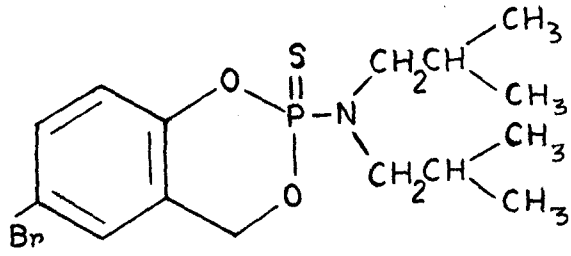


n-Butylamidophosphorodichloridothionate (0.01 mole, 2.06 gm), 5-chlorosaligenin (0.01 mole, 1.58 gm) and anhydrous potassium carbonate in 50 ml acetone were allowed to react. A white solid compound was obtained which was recrystallised from methanol, m.p. 163°C; Molecular formula $C_{11}H_{15}O_2NPSCl$; Mol. wt. 291.5; Yield 65-70%.

Analysis:

Found	: C, 45.21%; H, 5.10%; N, 4.76%
Calculated for $C_{11}H_{15}O_2NPSCl$	: C, 45.28%; H, 5.14%; N, 4.80%.

8.(iv) **2-N,N-diisobutylamido-6-bromo-4H-1,3,2-benzodioxaphosphorin-2-sulphide (BR-6):**



This compound (BR-6) was prepared by condensation of 2-hydroxy-5-bromobenzylalcohol (2.03g, 0.01 mole) and N,N-diisobutylamidophosphorodichloridothionate (2.62gm, 0.01 mole) in 50 ml acetone as solvent; K_2CO_3 was added by instalments to the stirred solution at 0° to 5°C. After an additional stirring (12-16h at the temperature 5-27°C), the solids were filtered off, and the solvent was removed under reduced pressure at room temperature. The crude product was washed with methanol saturated with n-heptane, and then the compound was recrystallize from hot methanol. 3.52 gm compound (BR-6) was obtained as a white crystalline solid.

Yield 90%; Mol. formula $C_{15}H_{23}O_2NPSBr$; m.p. 90°C; R_f 0.91 (benzene:acetone, 8:2) Mol. wt. 391.

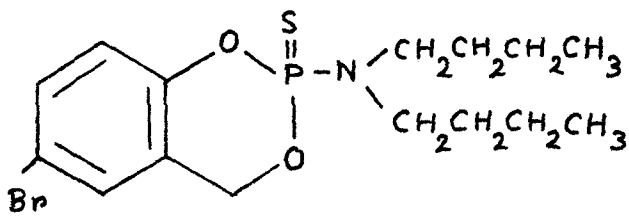
Analysis:

Found : **C, 46.18%; H, 5.89%; N, 3.59%**

Calculated for : **C, 46.0%; H, 5.88%; N, 3.58%**

$C_{15}H_{23}O_2NPSBr$

8.(v) 2-N,N-Dibutylamido-6-bromo-4H-1,3,2-benzodioxaphosphorin-2-sulphide (BR-10):



The condensation of 2-hydroxy-5-bromobenzyl alcohol (2.03 gm, 0.01 mole) and N,N-dibutylamidophosphorodichloridothionate (2.62 gm, 0.01 mole) in presence of K_2CO_3 (2.76 gm, 0.02 mole) in 50 ml acetone gave BR-10 as liquid, BR-10 was purified.

The crude product was washed with methanol saturated with n-heptane and then the compound was purified by column chromatography using silica gel (60-120 mesh) as absorbent. Elution was carried out with dry, distilled thiophene free benzene, and the progress of chromatographic fractionation had been monitored by examining the IR spectra of selected fraction and also by TLC technique (coating material silica gel G, solvent benzene : acetone, 9:1). After removal of solvent <u>in vacuo</u> 2.70 gm. of colourless liquid product was obtained.

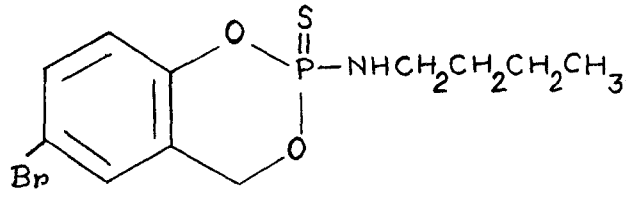
Yield 70%; Mol. formula $C_{15}H_{23}O_2NPSBr$; R_f 0.85 (benzene : acetone, 9:1); Mol. wt. 391.

Analysis:

Found : C, 46.21%; H, 5.90%; N, 3.60%;

Calculated for $C_{15}H_{23}O_2NPSBr$: C, 46.00%; H, 5.88%; N, 3.58%

8.(vi) **N-Butylamido-6-bromo-4H-1,3,2-benzodioxaphosphorin-2-sulphide (Br-24):**



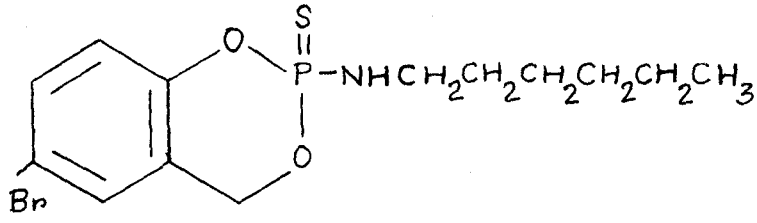
n-Butylamidophosphorodichloridothionate (0.01 mole, 2.06 gm), 5-bromo saligenin (0.01 mole, 2.03 gm) and anhydrous potassium carbonate (0.02 mole, 2.76 gm) in 50 ml acetone were allowed to react. A yellow solid compound was obtained after purification through silica gel column chromatography, m.p. 70°C; Molecular formula $C_{11}H_{15}O_2NPSBr$; Mol. wt. 335. Yield, 55-60%.

Analysis:

Found : C, 39.22%; H, 4.40%; N, 4.13%

Calculated for $C_{11}H_{15}O_2NPSBr$: C, 39.28%; H, 4.46%; N, 4.17%.

8.(vii) <u>2-Hexylamido-6-bromo-4H-1,3,2-benzodioxaphosphorin-2-sulphide (Br-27)</u>:



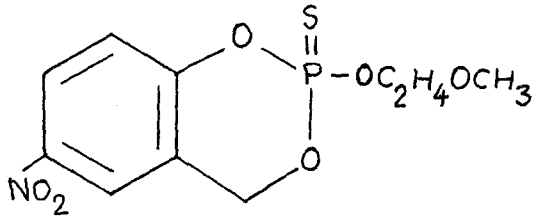
Hexylamidophosphorodichloridothionate (2.34 gm, 0.01 mole), 5-Bromosaligenin (2.03 gm, 0.01 mole) and anhydrous potassium carbonate (2.76 gm, 0.02 mole) in 50 ml dry acetone gave a liquid.

Yield 45-50%; Molecular wt., 363; Molecular formula, $C_{13}H_{19}O_2NPSBr$.

Analysis:

Found	: C 42.90%; H 5.17%; N 3.78%;
Calculated for $C_{13}H_{19}O_2NPSBr$	: C 42.86%; H 5.21%; N 3.85%.

8.(viii) <u>2-(β-methoxy)ethoxy-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-sulphide (BD-1)</u>:

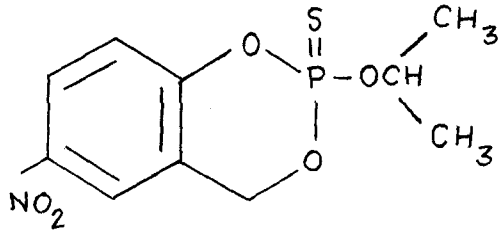


5-Nitro-saligenin (3.2 gm, 0.019 mole), (β-methoxy) ethyldichloridophosphorethionate (4.0 gm, 0.019 mole) and anhydrous potassium carbonate (5.2 gm, 0.038 mole in 50 ml dry acetone gave 2-(β-methoxy) ethoxy-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-sulphide (4.5 gm), 80% yield; as a white crystalline solid; m.p. 84°C; Molecular formula $C_{10}H_{12}O_6NPS$; Mol. wt. 305.

Analysis:

Found	: C 39.35%; H 4.0%; N 4.60%
Calculated for $C_{10}H_{12}O_6NPS$	: C 39.34%; H 3.96%; N 4.59%.

8.(ix) <u>2-isopropoxy-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-sulphide (BD-5)</u>:

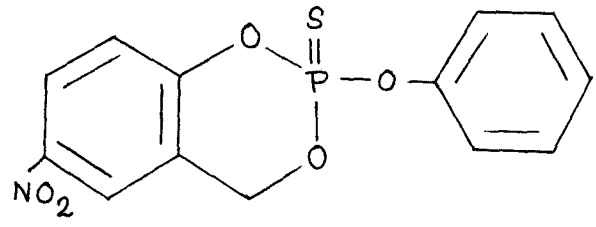


5-nitro saligenin (5.3 gm, 0.031 mole) i-$C_3H_7OP(S)Cl_2$ (6 gm) and K_2CO_3 (8.5 gm) gave BD-5 (4.8 gm, 55% yield) as white crystals; m.p. 82-83°C; R_f 0.63; Mol. wt. 289; Molecular formula $C_{10}H_{12}O_5NPS$.

Analysis:

Found	: C 41.50%; H 4.20%; N 4.83%
Calculated for $C_{10}H_{12}O_5NPS$	: C 41.52%; H 4.15%; N 4.84%

8.(x) <u>2-Phenoxy-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-sulphide (BD-9)</u>:

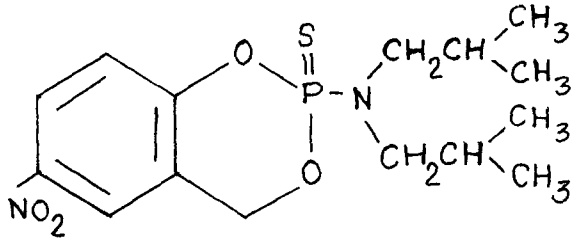


5-Nitro-saligenin (4.9 gm, 0.015 mole), $C_6H_5OP(S)Cl_2$ (3.4 gm) and K_2CO_3 (4.15 gm) gave 2-Phenoxy-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-sulphide (5 gm, 55% yield) as white crystals; m.p. 95°C; Molecular wt 323, Molecular formula $C_{13}H_{10}O_5NPS$.

Analysis:

Found	: C 48.28%; H 3.10%; N 4.25%
Calculated for $C_{13}O_{10}O_5NPS$	: C 48.30%; H 3.12%; N 4.33%

8.(xi) <u>2-N,N, Diisobutylamido-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-sulphide (BD-25)</u>:

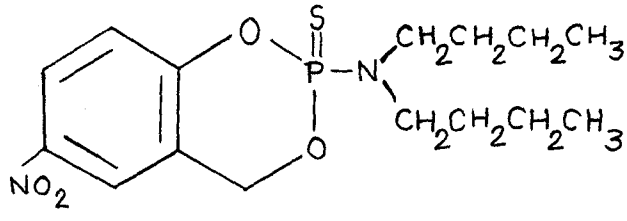


0.01 mole i.e. 1.69 gm of 5-nitrosaligenin 0.01 mole i.e. 2.62 gm of diisobutylamidophosphorodichloridothionate and 0.02 mole i.e. 2.76 gm of K_2CO_3 were allowed to react in 50 cc acetone and worked up as in (7). 2.6 gm (73.6% yield) solid white crystalline compound was obtained; m.p. 136°C ; Mol. wt. 358; Molecular formula $C_{15}H_{23}N_2O_4PS$.

Analysis :

Found	: C 50.29%; H 6.44%; N 7.88%
Calculated for $C_{15}H_{23}O_4N_2PS$	: C 50.27%; H 6.42%; N 7.82%.

8. (xii) <u>2-Dibutyl amido-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-sulphide (BD-29)</u>:



0.005 mole, 0.845 gm of 5-nitrosaligenin, 0.005 mole, 1.31 gm of dibutylamidophosphorodichloridothionate and 0.1 mole, 1.38 gm of potassium carbonate were allowed to react in 50 cc acetone. A solid white crystalline compound was obtained; m.p. 70°C; Yield 80-90%; Mol. wt. 358; Molecular formula $C_{15}H_{23}N_2O_4PS$.

Analysis:

Found	: C 50.28%; H 6.71%; N 7.88%
Calculated for $C_{15}H_{23}N_2O_4PS$	: C 50.27%; H 6.70%; N 7.82%.

9. Insecticidal tests:

9.(a) On Cockroaches (Periplaneta americana):

Oral insecticidal tests were performed on the Cockroach, Periplaneta americana (Linn.) according to Busvine[4] with minor modifications. Adults of P. americana, weighing about 0.8 gm to 1.2 gm, were collected from a particular location in the North Bengal University Campus. In the field, they were never exposed to any organophosphorus insecticides. For preliminary experiment, ten roaches in each pot were exposed to different doses of the compounds in dry sugar bait and after 24 hours the mortality was determined, and the minimum concentration required for 100 percent mortality (LC_{100} μg/gm body wt. basis) was found out.

To determine the more precise LC_{100} (the minimum concentration required for 100 percent mortality) value of each compound, one Cockroach of known weight in each pot was exposed to known quantity of the compounds, progressively increasing its concentration by 2 μg/gm, for Salithion the concentration was increased by 1 μg/gm. Each experiment was triplicated and the average LC_{100} value was found out by using the simple arithmetical procedure according to McIntosh[5]. Before conducting the experiments on them, the Cockroaches were kept starved for 24 hours. However, the varying susceptibility of male and female Cockroaches to different compounds were ignored during the experiment.

9.(b) On Grasshoppers, (Oxya nitidula (Walker)

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, 1989 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 mouth and thoracic region (ventral side) of the insect. Each test was triplicated.

For BD-5, BD-8 and Salithion the concentration was increased by 0.1 μg/insect, for BD-1 by 0.2 μg/insect and for other compounds the concentration was increased by 1 μg/insect.

9.(c) On Blow-fly (Chrysomaya megacephala):

Contact insecticidal activity tests on blow flies, C. megacephala were performed according to Eto et al[7]. Acetone solution of the individual compounds were topically applied to female adult insects. Two replicate samples each of ten insects were used for every treatment. The insects were kept at room

temperature (25-28°C). Mortality counts were determined after 24 hours of experiment.

9. (d) On Aphids:

Insects of standard age and weight were selected and starved for two hours, prior to the test, so as to ensure that they would consume the required amount of the treated leaves[8]. Ten different concentrations (1 μg/ml to 10 μg/ml) of each compound (BD-1, BD-5, BD-9, BD-25, BD-29, Cl-6, Cl-10, Cl-24, Br-6, Br-10, Br-24, Br-27) were prepared in acetone solution and 1% Tween-water mixture. Ten petridishes of equal sizes were taken and sterilized. Fresh leaves were placed in each petridish and they were then sprayed by the compound and acetone-Tween-water mixture. Before each spraying the equipments were thoroughl cleaned and dried. One control was prepared by spraying the leaves with only acetone-Tween-water mixture. The acetone in each petridish was evaporated by a dryer. After drying, ten Aphids (adult) were placed in each petridish (10 insects/petridish). The petridishes were covered with cellophane-paper tightly, and perforated so that the Aphids could breath properly. The dishes were properly marked and kept for observation. After 24 hours and 48 hours, the mortality counts were determined.

10. Acute oral toxicity tests on Rats:

Acute oral toxicity testing was conducted on 6-12 months old male white albino rats, weighing 140-200 gm, each housed in separate compartment of a cage. All animals had free access to

diet including water. Different dosages of a compound were mixed with boiled goat liver and given to the animals at their habitual feeding time[9]. The animals were observed and 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 proportionally. The negligible amount of compound washed by the animal during dieting was roughly accounted for in determining the dosage.

11. Acute oral toxicity and delayed neurotoxicity in Hens:

White leghorn hens (weighing about 1.1 to 1.3 kg) were purchased from local market; they were kept at room temperature and had free access to diet. The compounds were administered orally (by using flour capsule) at the following dosages.

(i) the isopropoxy compound (BD-5) - 100, 160 and 200 mg/kg (Table - 15).

(ii) the methoxy compound (BD-8) - 50, 100 and 150 mg/kg (Table - 16).

(iii) N,N, Diisobutyl amido (CL-6) - 100, 150 and 200 mg/kg (Table -17).

(iv) N,N, Diisobutyl amido (BR-6)- 100, 200 and 300 mg/kg (Table - 18).

The animals were observed for 10 days. The toxic symptoms were recorded and the LD_{50} value was calculated[10].

The delayed neurotoxicity study was carried out according to the proposed guide line of the Environmental Protection Agency

of the United States[11]. The compounds were administered orally to the specified number of hens at the dosages mentioned in the Table (15 to 18). To protect the animals from acute intoxication, atropine sulphate (0.54 mg/kg body wt by subcutaneous injection) was administered several times depending on the symptoms; for example in the case of N,N, Diisobutyl compound (Cl-6), atropine sulphate was injected eight times at the interval of about 1 hour .

Ten days after the first administration of the compounds, the same treatment was repeated and the hens were observed for six weeks. During the whole six weeks experimental period, paralysis in legs were checked as an indicator for the delayed neurotoxicity. After the observation period, all the treated birds excluding those dead of acute intoxication were sacrificed and their sciatic nerves were dissected out and examined histopathologically through normal microtechnique procedure (using Bouin's fluid as the fixative, paraffin for embedding, hematoxylin and eosin as stains). Five μ thick sections were cuted.

12. Anticholinesterase activity:

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

12.(a) 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 by colorimeter method of Kramer and Gamson[12,13], using indophenyl acetate as an internal substrate indicator in 0.05M phosphate buffer of pH 8.0. The reaction mixture contained 5 ml of enzyme-buffer solution (4.8 ml of 0.05 M phosphate buffer solution of pH 8.0 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 9.6×10^{-5} M). The reading of 'control' and 'sample' were taken at 625 nm, after exactly 30 min. incubation.

Materials and Method

12(a)(i) Materials:

(1) Goat whole blood:

150 ml fresh blood was collected from goat and mixed with 15 mg ammonium oxalate (anti coagulating agent) in a 250 ml standard joint bottle and was shaken well. The bottle containing blood was then kept in the freezer at $0^{\circ}C$.

(2) Buffer solution (0.05M Potassium dihydrogen phosphate):

Clark and Lub's Buffer of pH 8.0. Five hundred ml of 0.1M KH_2PO_4 solution was mixed with 475 ml of 0.1N NaOH solution and diluted to 1 litre after the pH was adjusted to 8.0.

(3) Glycerol solution:

Ten ml of glycerol was diluted to 100 ml with absolute alcohol.

(4) Indophenyl acetate:

Indophenyl acetate (0.008g) was dissolved in 10 ml of absolute ethyl alcohol (3.3×10^{-3}M solution). Thus the final concentration of indophenyl acetate in the reaction mixture was 9.6×10^{-5}(M).

(5) Saline solution (0.9%):

Nine gms of NaCl was dissolved in one litre of distilled water.

(6) Salt solution:

$MnCl_2$ (2.03 gms) and NaCl (2.15 gms) were dissolved in 250 ml of water.

12.(a)(ii) Method:

The standard incubation mixture contained 5 ml of the enzyme solution, 0.5 ml of glycerol solution and 0.15 ml of indophenyl acetate solution (final concentration 9.6×10^{-5}M). Each organophosphorus compound as an inhibitor, was added as acetone solution, in the reaction mixture, acetone in the reaction mixture was removed by blowing air. The reaction mixture containing the enzyme and glycerol solutions with or without organophosphorus compound (control) were kept at room temperature ($26^{\circ}C \pm 1^{\circ}C$) for 30 minutes and then the indophenyl acetate solution as substrate was added. The incubation was carried out

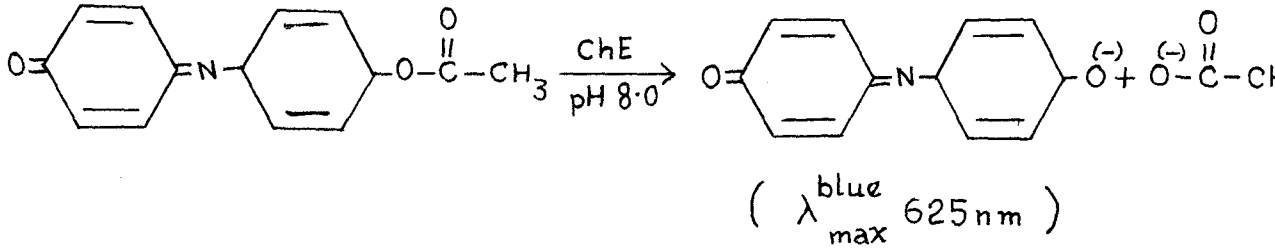
at 30°C for 30 mins. After incubation the optical density of the reaction mixture was measured at 625 nm by the use of Shimadzu UV-240 Spectrophotometer.

Calculation :

$$\% \text{ Inhibition} = \frac{\text{Absorbance (Con)} - \text{Absorbance (sample)}}{\text{Absorbance (Con)}} \times 100$$

12(b) Anticholinesterase activity in Blow-fly head homogenate:

The method employed to determine the inhibition of the activity of acetylcholinesterase in blow-fly head homogenate (BFAChE) by the organophosphorus compounds was the colorimetric method of Kramer and Gamson[12,13] using the indophenylacetate as an internal substrate indicator in 0.05M phosphate buffer of pH 8.0. The enzymatic reaction for indophenyl acetate is as follows:



Materials and Method:

12.(b)(i) Materials:

Preparation of working solution of acetylcholinesterase from blow-fly heads:

The heads obtained from 250 flies (Chrysomeya megacephala) were homogenized in 2 ml of salt solution by using size No. -1 morter (prechilled) containing 2 gm of sand. The homogenates were transferred in to 50 ml of plastic centrifuge tube by washing with 3 ml of saline and 10 ml of buffer solutions, respectively. After centrifugation at 10,000 r.p.m. for 10 min at 4°C, the supernatant fraction was obtained by decantation into a graduated cylinder. On the other hand, the precipitates were resuspended in 10 ml of buffer solution. The suspension obtained was recentrifuged by the same procedure as mentioned above. This enzyme extraction procedure was repeated twice. After each supernatant fraction obtained here was combined, its final volume was adjusted to 250 ml with buffer solution (equivalent to 1 fly head/ml solution). This solution was stored in deep freezer as the stock solution. When the stock solution was used as the enzyme source for cholinesterase activity measurement, the solution was diluted with 4 volumes of buffer solution (equivalent to 1 fly head/5 ml solution).

The other reagents such as indophenylacetate, phosphate buffer, glycerol solutions were prepared as described in 12(a)(i).

(ii) Method:

The method and calculation for the determination of the inhibition of acetylcholinesterase in Blow-fly head homogenate were the same as described in 12(a)(ii).

13. Antifungal activity:

Helminthosporium oryzae and Pyricularia oryzae were employed to determine the antifungal activity by using poisoned food technique[14]. Desired volume of acetone solution of Phosphoramidothionate was mixed with water containing 0.2% Triton-X and the solution was mixed with melted malt agar so as to get the desired concentration of the compound in the media. The test medium was then poured in to sterile petridishes and after solidification the 7 mm 8 days old culture disc was placed asceptically at the centre of the petridishes. Three replications of each test with appropriate control under same conditions were maintained. These petridishes were incubated at 26 ± 1°C and the diameter of the colony was measured after 24 hours, 48 hours, 72 hours and 96 hours in both the cases. Percent inhibition of the growth of each colony over control was calculated following the equation given by Vincent[15].

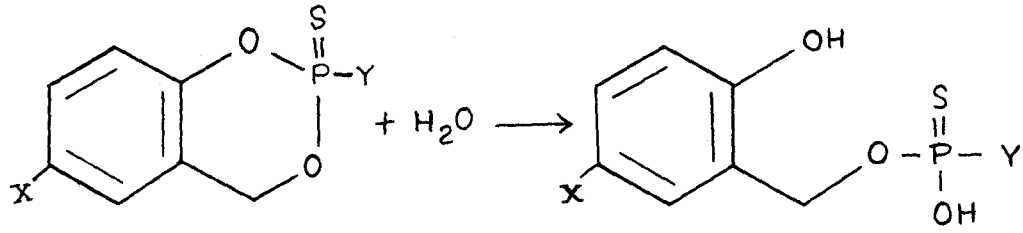
14. Phytotoxicity Test:

Phytotoxicity was conducted according to the procedure of Eto et al[16]. Acetone solution of the compounds mixed with fixed amount of water containing 0.2% Triton-X was prepared. Five ml of this aqueous suspension containing 500, 250 or 100 ppm of each phosphoramidothionate was poured in to a petridish, the bottom of which was covered with absorbent cotton. Ten seeds of wheat (Triticum sp. U262 variety supplied by the National Seed Corporation of India) were placed on the cotton and kept at room temperature (25° - 27°C) for five days. Water (2-4 ml) was added occasionally to each petridish so that the seeds remained

in moist conditions. Each test was triplicated. Number of germinations were counted after 5 days.

15. Chemical hydrolysis:

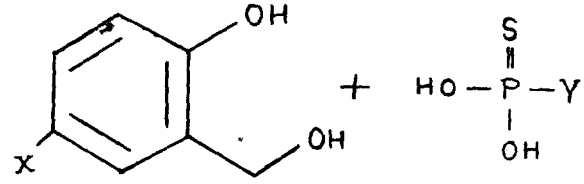
Since the compounds are analogous to Salithion[17], it is envisaged to proceed with the initial fission of aryl ester bond in dioxaphosphorin ring followed by the liberation of 5-nitro/chloro/bromo saligenin.



where,

X = NO_2, Cl, Br

Y = $-N\begin{matrix}R_1\\R_2\end{matrix}$, OR



The chemical hydrolysis studies of phosphoramidothionates have been studied in 9.5 mM NaOH solution in 50% ethanol (pH 11.85) and that of alkoxy compound the experiments performed with phosphate buffer in 20% ethanol solution (pH 7.7) at 30°C.

Determination of the hydrolytic constants of the compounds involve the following stages:

(i) Determination of molar extinction co-efficient (ϵ_1) of 5 nitro/chloro/bromo saligenin in the alkali solution (9.5 mM NaOH in 50% ethanol, pH 11.85).

A weighed amount of pure 5-nitro/chloro/bromo saligenin was dissolved in a required volume of alkaline solution and extinction coefficient values of its corresponding to wave lengths 400, 410, 420 nm (for 5-nitro saligenin) and 294, 298, 300 nm (for 5-chloro/bromo saligenin) were found out by measuring the absorbance in Beckmann DU-2 Spectrophotometer. The molar extinction coefficient of nitro saligenin have also been determined by the above mentioned method in phosphate buffer.

(ii) Determination of the molar extinction co-efficient (ϵ_2) of the 6-chloro/bromo saligenin cyclic phosphoramidothionates in the same alkaline solution (pH 11.85).

As the rate of hydrolysis of the 6-chloro/bromo saligenin cyclic phosphoramidothionates were extremely slow, the optical densities (at λ= 294, 298 and 300 nm) of the alkali solution of any of the phosphoramidothionates of known concentration were measured immediately after the preparation of the solution, the optical densities and the concentration of the phosphoramidothionates, the molar extinction coefficients of the phosphoramidothionates (ϵ_2) were determined.

(iii) Determination of the amount of compounds hydrolyzed (C_t) after a certain interval.

One ml of compound solution in absolute ethanol (Conc. 10^{-4}M) was added to 9 ml of the alkali solution (pH 11.85), so

that the final concentration of the compound was of the order of 10^{-5}M, and the stopwatch started. After suitable time interva the absorbance at 400, 410, 420 nm for nitro compounds and at 294, 300 nm for chloro and bromo compounds were measured in the same spectrophotometer.

The concentration of the phosphoramidothionates hydrolyzed (C_t) were calculated from the equation:

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

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

The Pseudo first order rate constants of the different compounds were calculated from the first order rate equation

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

where, C_o = initial concentration of the compound.

C_t = Concentration of the compound hydrolysed after time t.

The average pseudo first order rate constant (K_{hyd}) of a compound was computed from the rate constant values calculated by the least square regression analysis at each wave length. The half life periods of the different compounds were also calculated.

PART - II

RESULTS AND DISCUSSION

1. Synthesis:

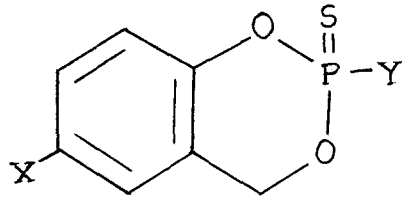
In our laboratory Das *et al*[18] prepared 6-nitrosaligenin cyclic amidophosphorothionates in solid form with high yield at low temperature. The method of Eto *et al*[19] using potassium carbonate and copper powder at elevated temperature in benzene was also employed; however, this method gives a pastey material from which it is very difficult to obtain pure compound.

We, however, succeeded in synthesizing several 2-alkyl amido/alkoxy-6-chloro/bromo/nitro saligenin cyclic phosphorothionates by the reaction of the appropriate alkyl amido/alkyl phosphorodichloridothionate with 5-chloro/bromo/nitro saligenin using K_2CO_3 as the dehydrogen chloride agent at low temperature 0-5°C.

We prepared some new compounds according to Das *et al*[18] at low temperature in our laboratory. The compounds viz. CL-6, CL-24, BR-6, BR-24, BD-1, BD-5, BD-9, BD-25 and BD-29 were obtained in the solid form and the compounds viz. CL-10, BR-10 and BR-27 were liquid in nature.

Table - 1

Percent yield and m.p. of different 6-chloro/bromo/ nitro saligenin cyclic phosphorus compounds



Code No.	X	Y	Yield(%)	m.p. (^{o}C)
CL-6	Cl	N,N-Diisobutylamido	78	91
CL-10	Cl	N,N-Dibutylamido	75	liquid
CL-24	Cl	N-Butylamido	65-70	163
BR-6	Br	N,N-Diisobutylamido	90	90
BR-10	Br	N,N-Dibutylamido	70	liquid
BR-24	Br	N-Butylamido	55-60	70
BR-27	Br	N-Hexylamido	45-55	liquid
BD-1	NO_2	Methoxy-ethoxy	80	84
BD-5	NO_2	Isopropoxy	55-60	82
BD-9	NO_2	Phenoxy	55-60	95
BD-25	NO_2	N,N-Diisobutylamido	80-90	135
BD-29	NO_2	N,N-Dibutylamido	80-90	70

2. SPECTRAL PROPERTIES:

The structures of the compounds have been determined by chemical analysis and UV, IR, Mass and NMR spectra. The analytical data along with the physical characteristics have been presented in this section. Spectral data are given below.

(a) SPECTRAL DATA:

(i) 2-N,N-Diisobutylamido-6-chloro-4H-1,3,2-benzodioxaphosphorin 2-sulphide (CL-6):

UV (Fig. 7)

λ_{max}^{EtOH} = 279 (ϵ = 1316)

IR (Fig. 8)

1010 cm^{-1} (s)	P-O-C (alkyl)
1240 cm^{-1} (s) and 915 cm^{-1} (s)	P-O-C (aryl)
815 cm^{-1} (s)	P = S (I)
650 cm^{-1} (s)	P = S (II)
1050 cm^{-1} (s)	Ar-Cl
735 cm^{-1} (s)	P-N str.

Mass (Fig. 9)

m/z	% RI
349 $(M + 2)^+$	6.79
347 (M^+)	16.83
314	22.23
304 (Base peak)	100.00

248	95.88
219	20.00
187	25.00
174	10.00
140	12.5
112	6.25
77	17.5

^{1}H NMR δ (Acetone d_6/TMS) ppm (Fig. 10)

0.73-0.90	(12H, doublet, four $-CH_3$ group)
1.20-2.20	(2H, multiplet, two CH group)
2.73-3.70	(4H, multiplet, $-N(CH_2)_2$ group)
4.83-5.66	(2H, multiplet, $-CH_2-O-P$ group)
6.80-7.30	(3H, aromatic hydrogen)

^{31}P NMR (Fig. 11)

δ ($CDCl_3/H_3PO_4$) ppm

69.80	(J, 10.79 H_z)

^{13}C NMR ($CDCl_3$/TMS) ppm (Fig. 12)

^{13}C atom	δ Values (ppm)	$^{n}J_{AB}$*	(Coupling constant Magnitude in H_z)
C_3	19.91 19.83	$^{4}J(^{31}P\text{-}N\text{-}C_1\text{-}C_2\text{-}C_3)$	7.32
C_2	25.72	$^{3}J(^{31}P\text{-}N\text{-}C_1\text{-}C_2)$	15.19
C_1	52.71 52.68	$^{2}J(^{31}P\text{-}N\text{-}C_1)$	3.00
C_4	66.35	$^{2}J(^{31}P\text{-}O\text{-}C_4)$	5.33
C_8	120.21	$^{3}J(^{31}P\text{-}O\text{-}C_9\text{-}C_8)$	8.22
C_5	124.97	-	-
C_{10}	122.24 122.14	$^{3}J(^{31}P\text{-}O\text{-}C_9\text{-}C_{10})$	10.81
C_7	128.34	-	-
C_6	128.98	-	-
C_9	149.68 149.61	$^{2}J(^{31}P\text{-}O\text{-}C_9)$	7.34

* $^{n}J_{AB}$ is used to represent a coupling over n bonds between nuclei A and B.

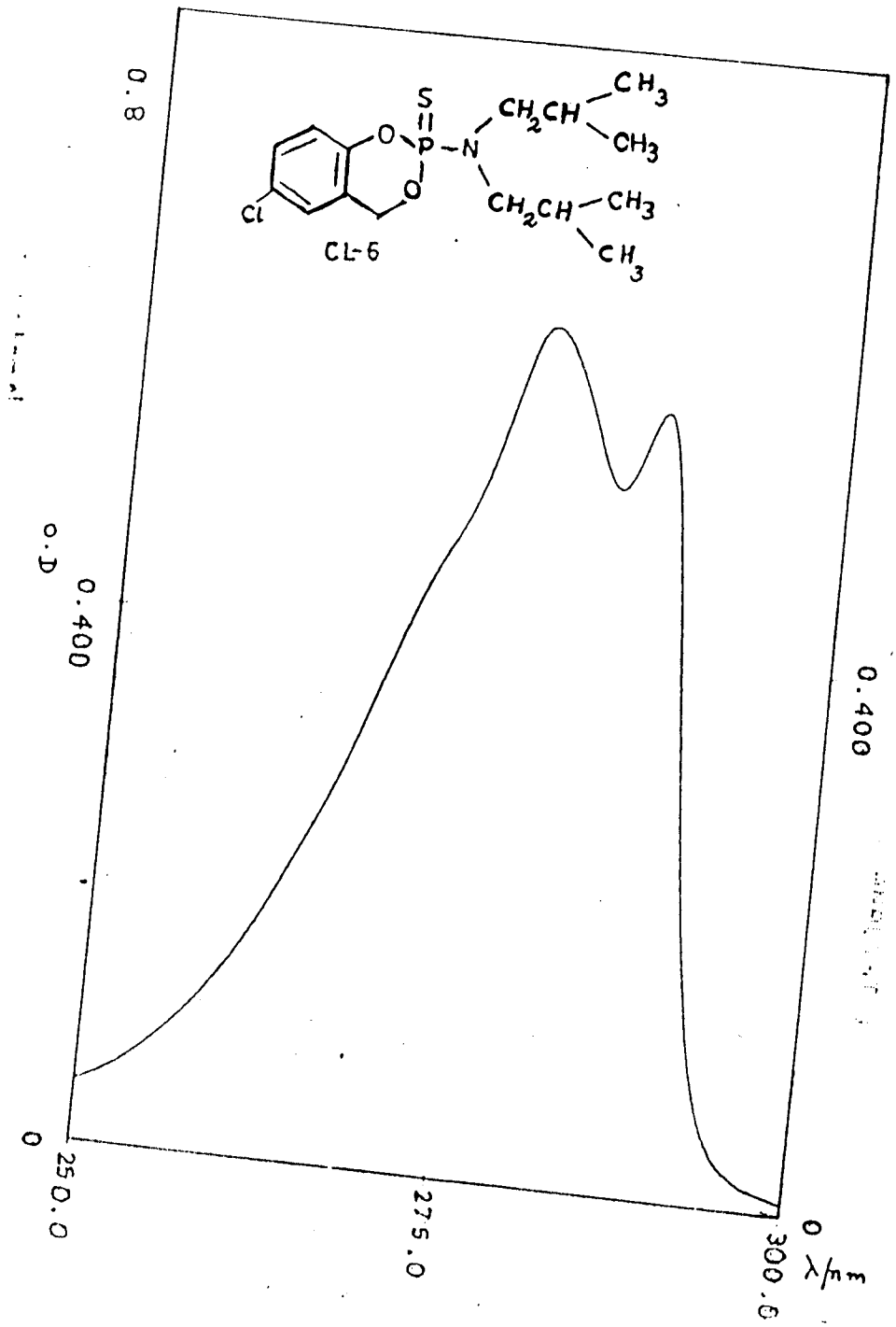


Fig.7 UV spectrum of 2-N,N-Diisobutylamido-6-chloro-4H-1,3,2-benzodioxaphosphorin 2-sulphide (Cl-6)

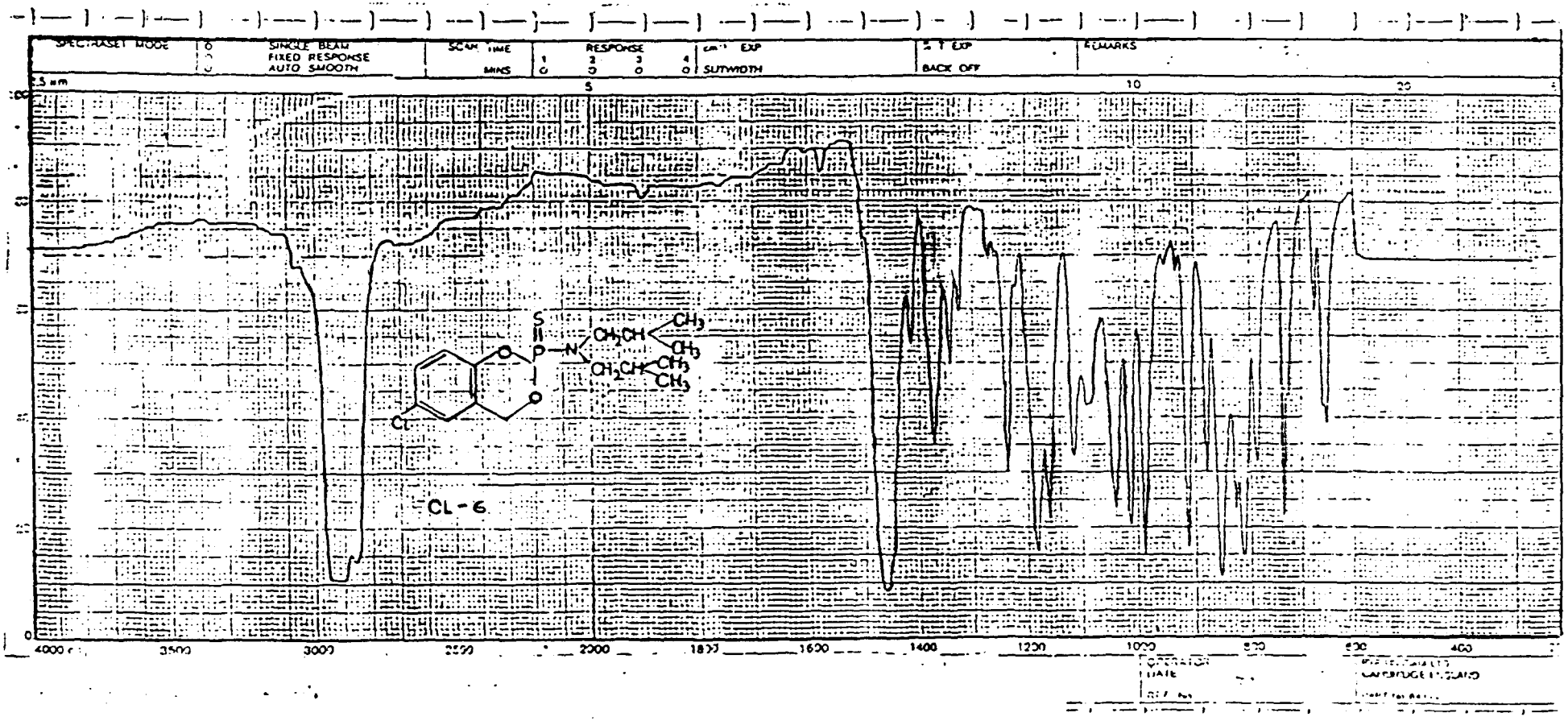


Fig. 8 IR spectrum of 2-N,N-Diisobutylamido-6-chloro-4H-1,3,2-benzodioxaphorin 2-sulphide (Cl-6)

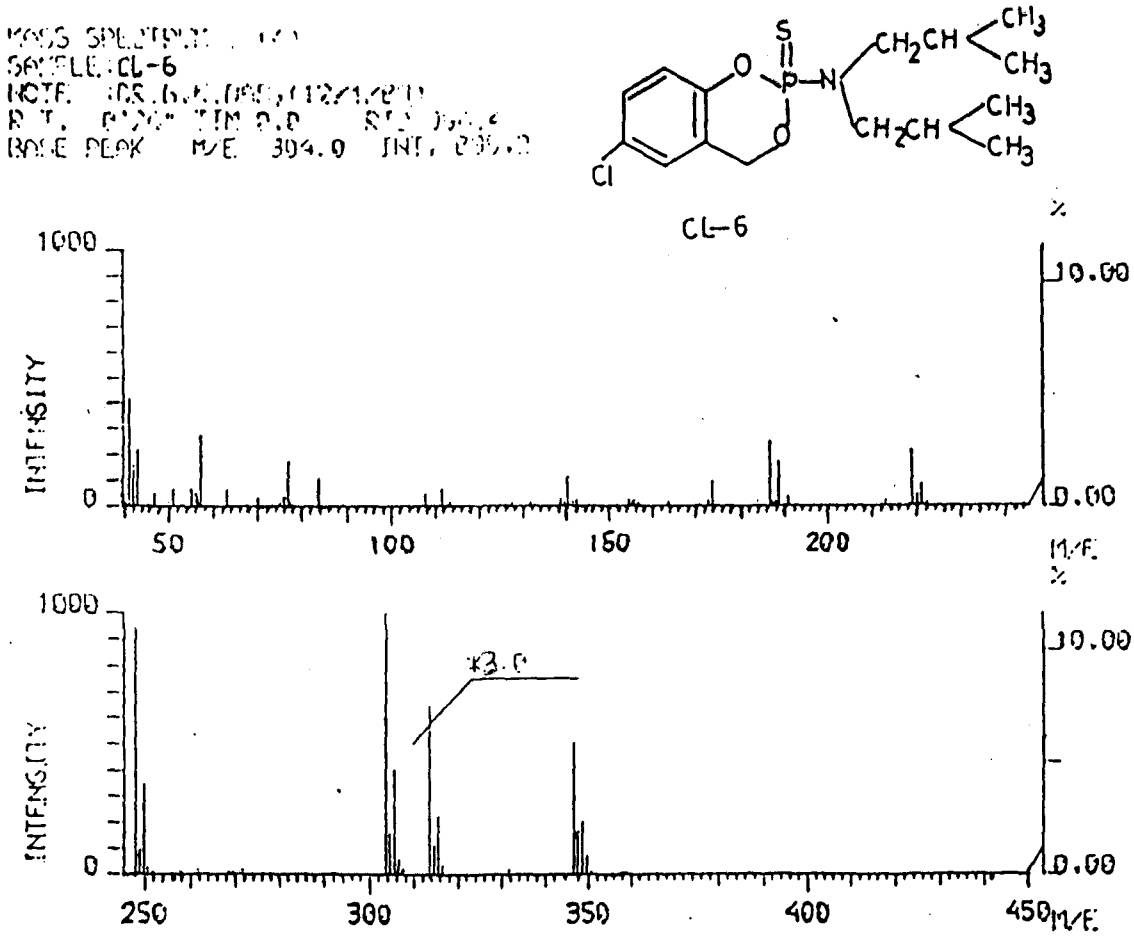


Fig. 9 Mass spectrum of 2-N,N-Diisobutylamido-6-chloro-4H-1,3,2-benzodioxaphosphorin 2-sulphide (CL-6).

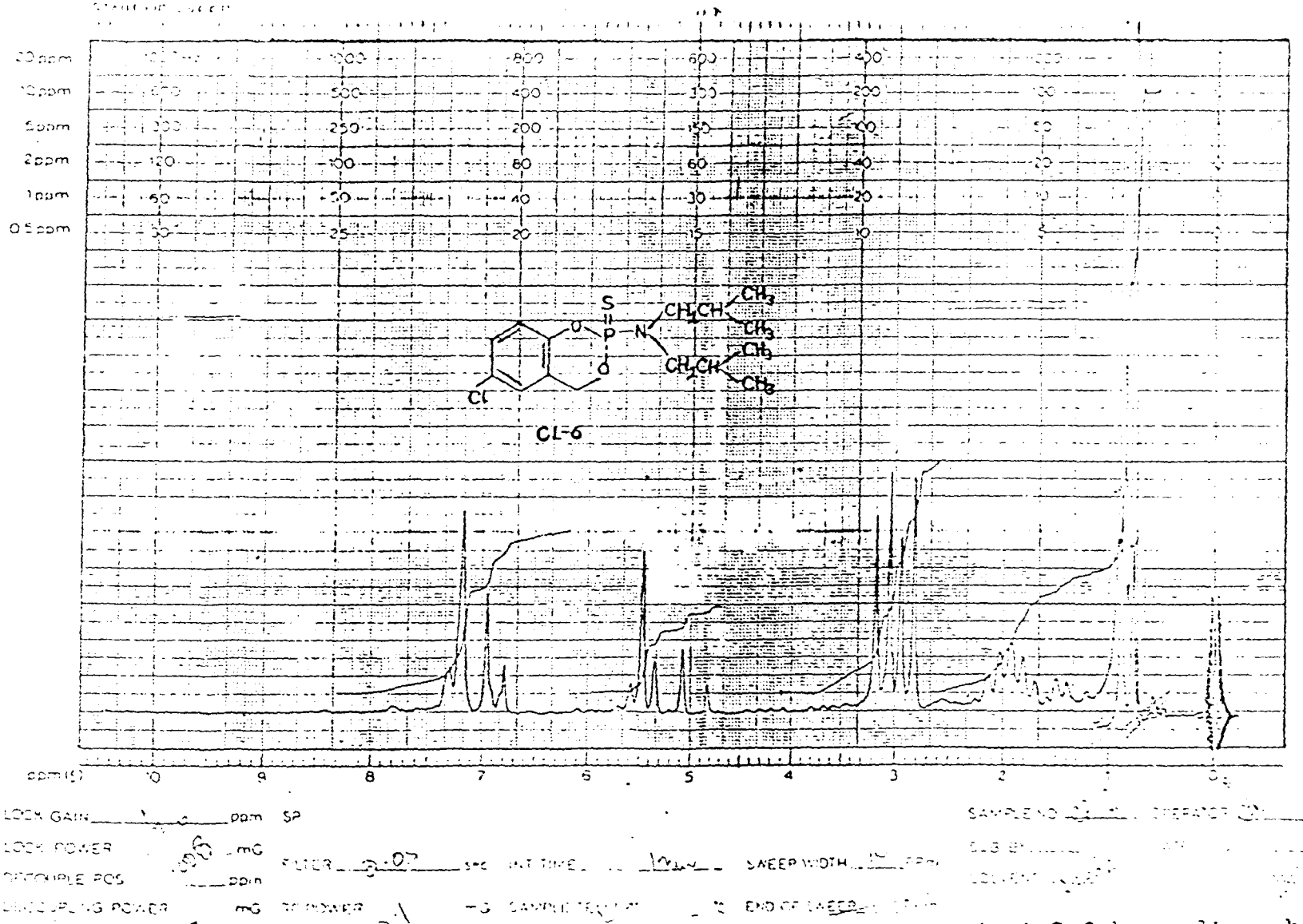
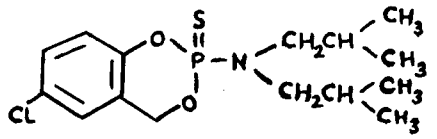
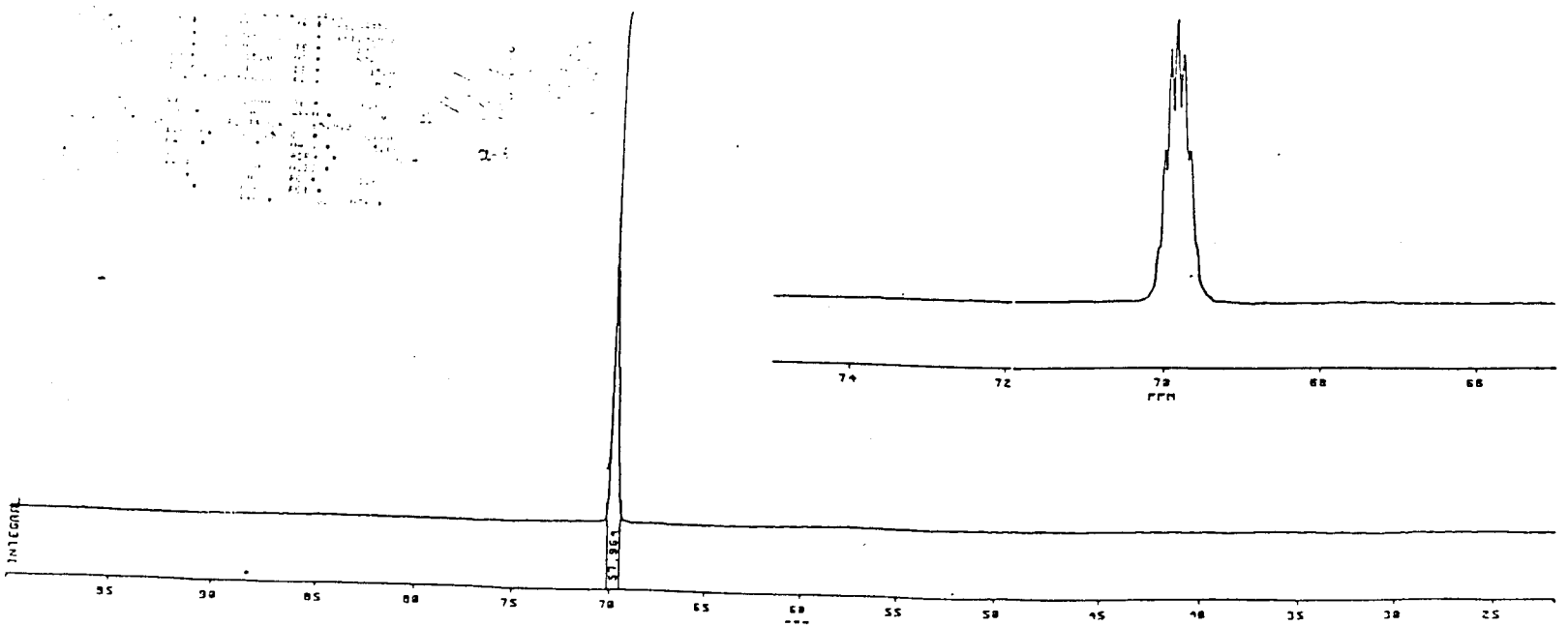
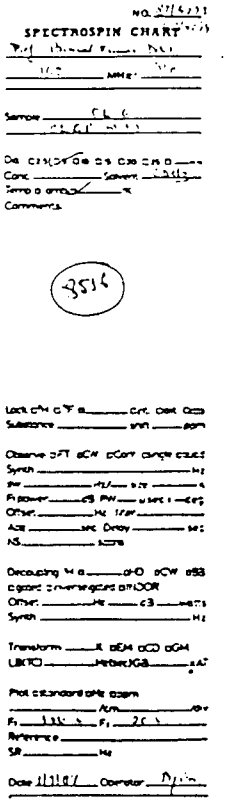


Fig.10 ^{1}H NMR spectrum of 2-N,N-Diisobutylamido-6-chloro-4H-1,3,2-benzodioxaphosphorin 2-sulphide (CL-6)

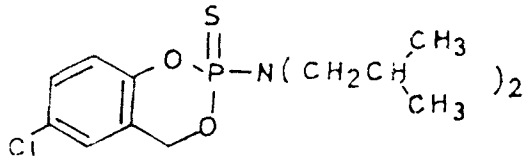


CL-6





Fig,11.. ^{31}P N.M.R. spectrum of 2-N, N-Diisobutylamido-6-chloro-4H-1, 3, 2-benzodioxaphosphorin 2-sulphide,



CL-6

Fig. 12 ^{13}C N.M.R. spectrum of 2-N, N-Diisobutyl amido-6-chloro-4H-1, 3, 2-benzodioxaphosphorin 2-sulphide.

(11) **2-N,N-Dibutylamido-6-chloro-4H-1, 3,2-benzodioxaphosphorin 2-sulphide (CL-10):**

UV (Fig. 13)

λ_{max}^{EtOH} = 279 nm (ϵ = 1620)

IR (Fig. 14)

1020 cm^{-1} (s)	P-O-C (alkyl);
1240-1250 cm^{-1} (s) and 910 cm^{-1} (s)	P-O-C (aryl);
810 cm^{-1} (s)	P = S (I);
655 cm^{-1} (s)	P = S (II);
1040 cm^{-1} (s)	Ar-Cl;
730 cm^{-1} (s)	P-N str.

Mass (Fig. 15)

m/z	% RI
349 $(M + 2)^+$	6.58
347 (M^+)	19.05
314 (Base peak)	100.00
304	25.20
262	33.80
248	24.90
219	19.17
187	37.50
174	37.50
140	15.00
112	25.00
77	37.50

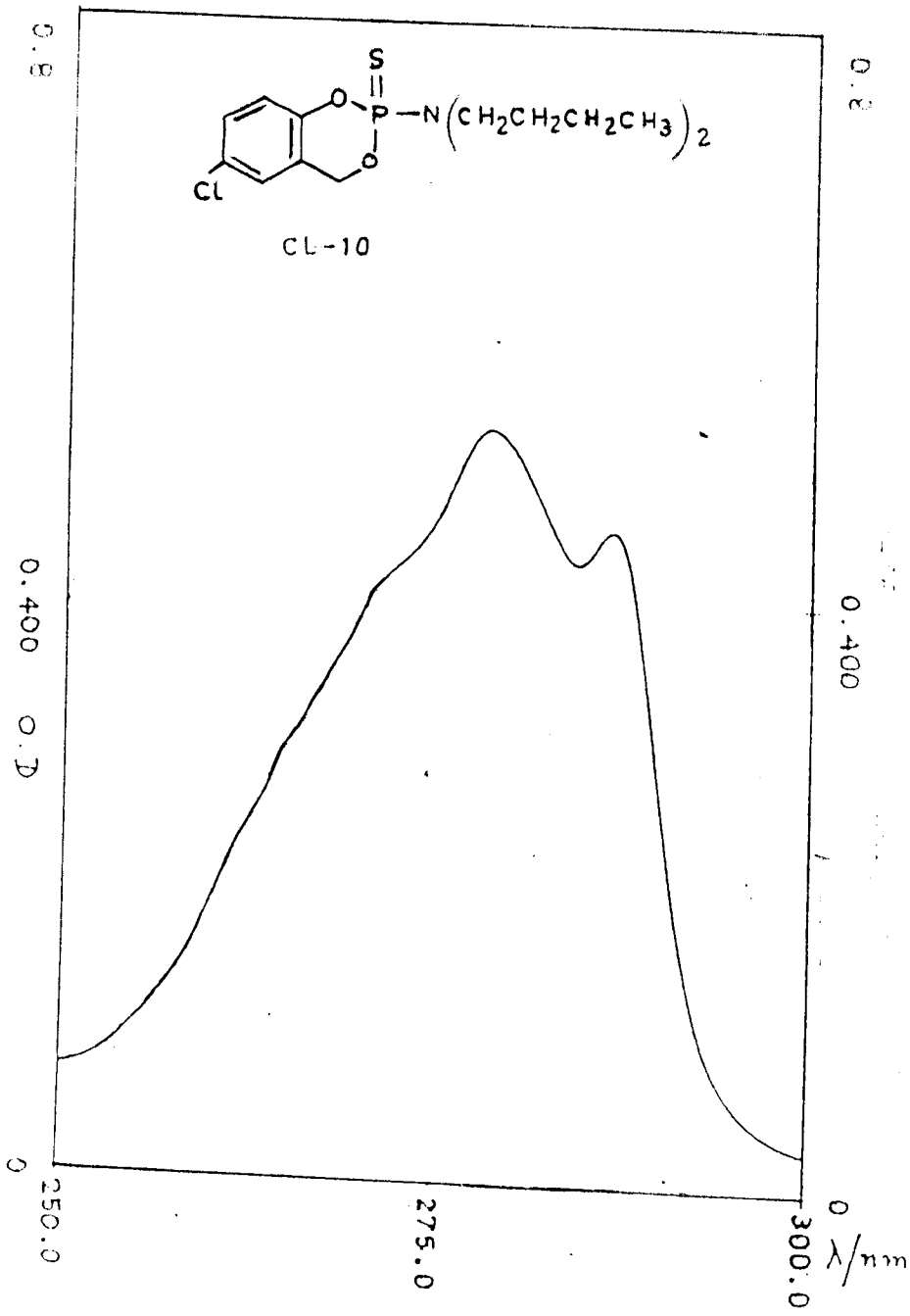


Fig.13 UV spectrum of 2-N,N-Dibutylamido-6-chloro-4H-1, 3,2-benzodioxaphosphorin 2-sulphide.

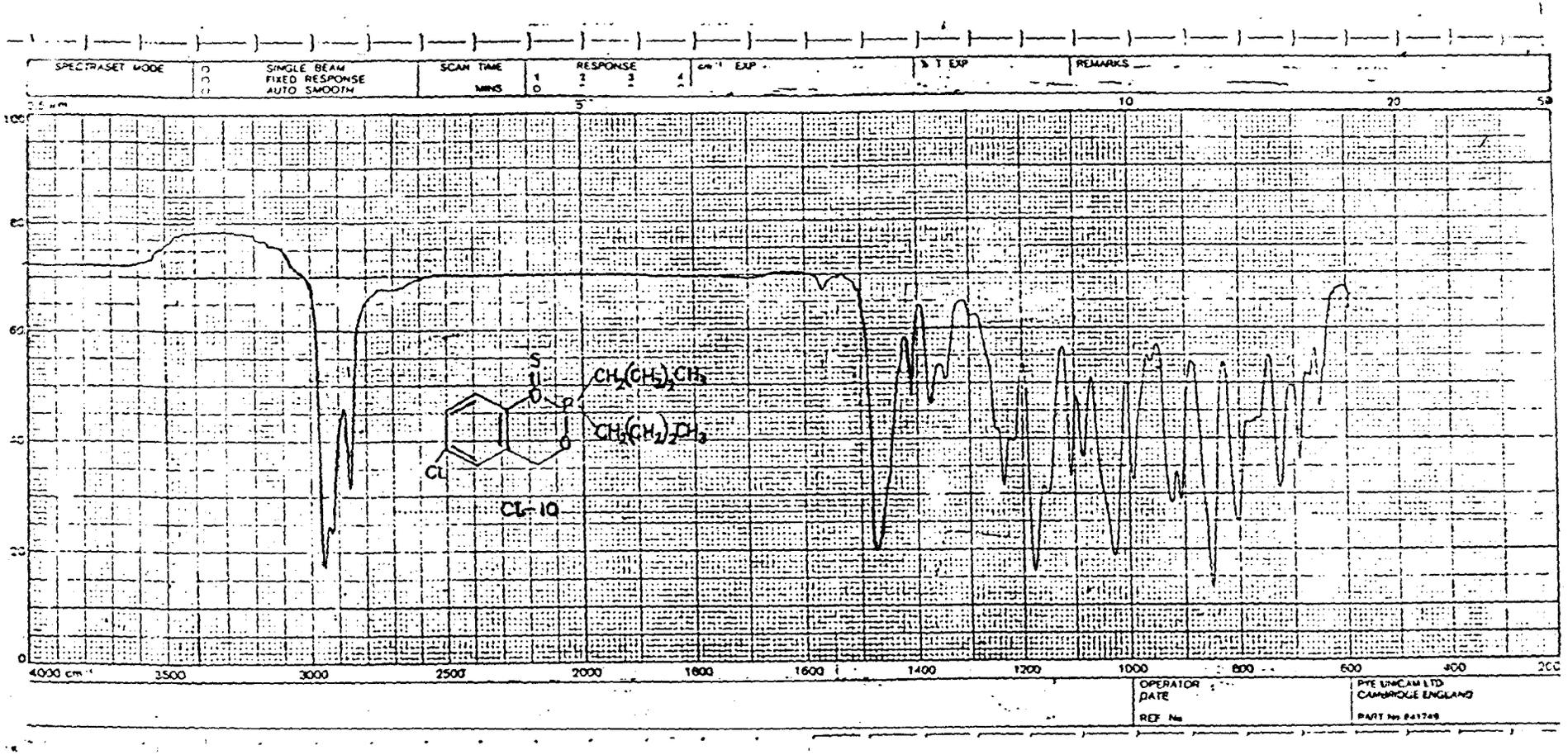


Fig.14 IR spectrum of 2-N,N-Dibutylamido-6-chloro-4H-1,3,2-benzodioxaphorin 2-sulphide (CL-10)

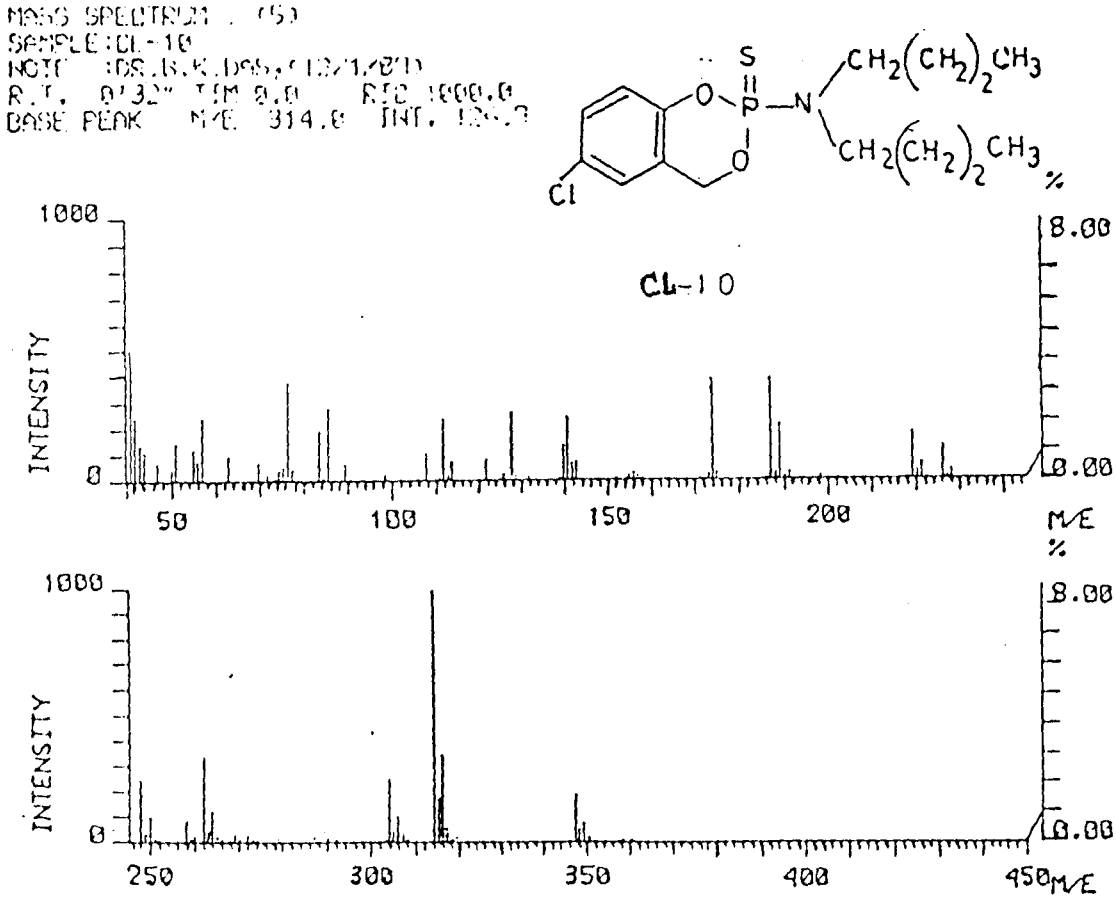


Fig.15 Mass spectrum of 2-N, N-Dibutylamido-6-chloro-4H-1,3,2-benzodioxaphosphorin 2-sulphide.

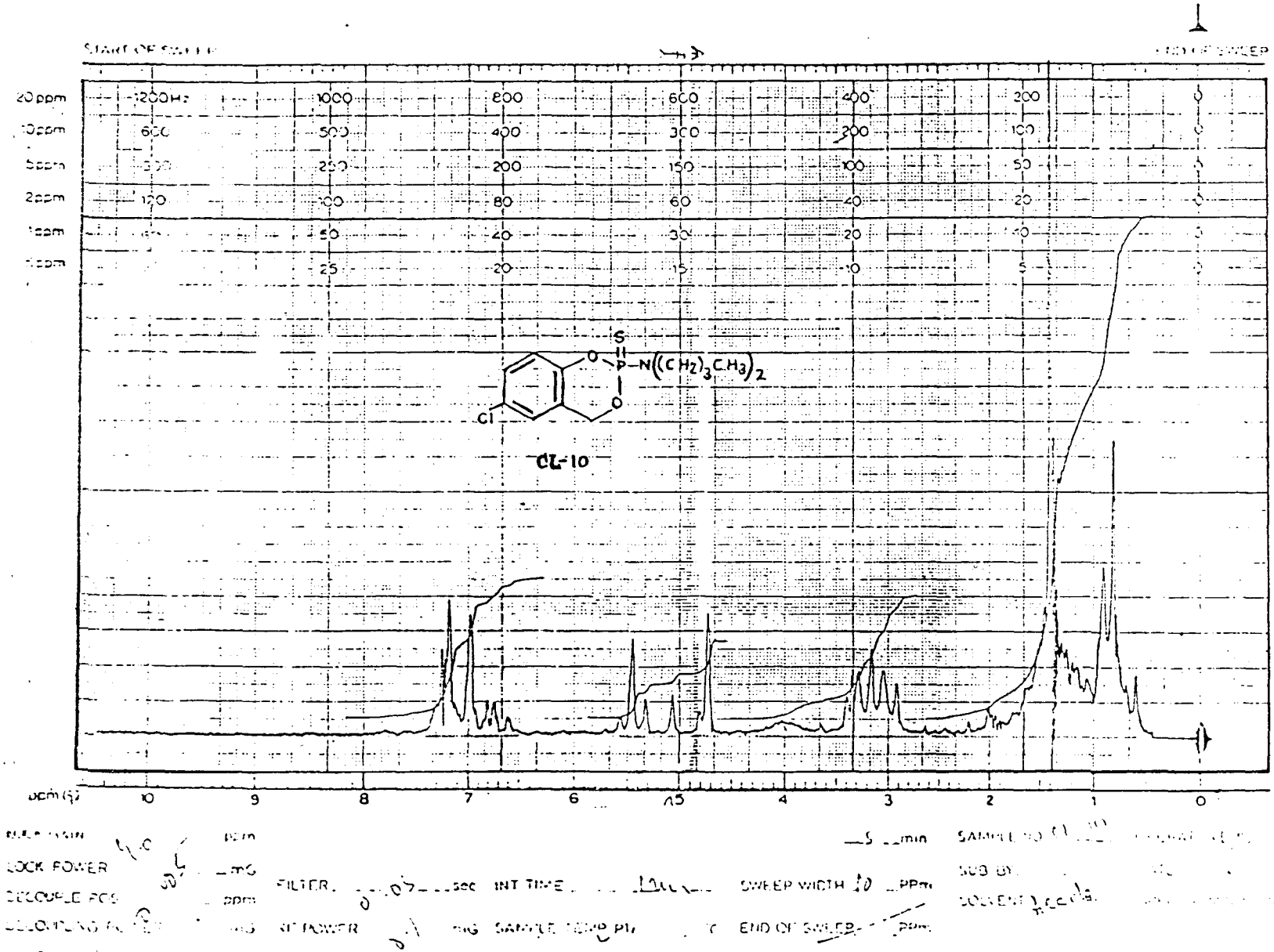


Fig.16 ^{1}H NMR spectrum of 2-N,N-Dibutylamido-6-chloro-4H-1,3,2-benzodioxaphosphorin 2-sulphide (CL-10)

^{1}H NMR δ (Acetone - d_6/TMS) ppm (Fig. 16)

0.80-0.90	(6H, triplet due to two $-CH_3$ group)
1.20-2.10	(8H, multiplet, due to two $-CH_2-CH_2-$ groups)
2.91-3.40	(4H, multiplet, $-N(CH_2)_2$)
4.73-5.56	(2H, multiplet, $-CH_2-O-P$)
6.80-7.46	(3H, multiplet, aromatic hydrog

(iii) 2-N-Butyl amido-6-chloro-4H-1,3,2-benzodioxaphosphorin 2-sulphide (CL-24):

UV (Fig. 17)

λ_{max}^{EtOH} = 283 nm (ϵ = 4268)

IR (Fig. 18)

1050 cm^{-1} (s)	P-O-C (alkyl)
1240 cm^{-1} (s) and 885 cm^{-1} (s)	P-O-C (aryl)
830 cm^{-1} (s)	P = S (I)
650 cm^{-1} (s)	P = S (II)
730 cm^{-1} (s)	P-N str.
3320 cm^{-1} (s)	N-H str.

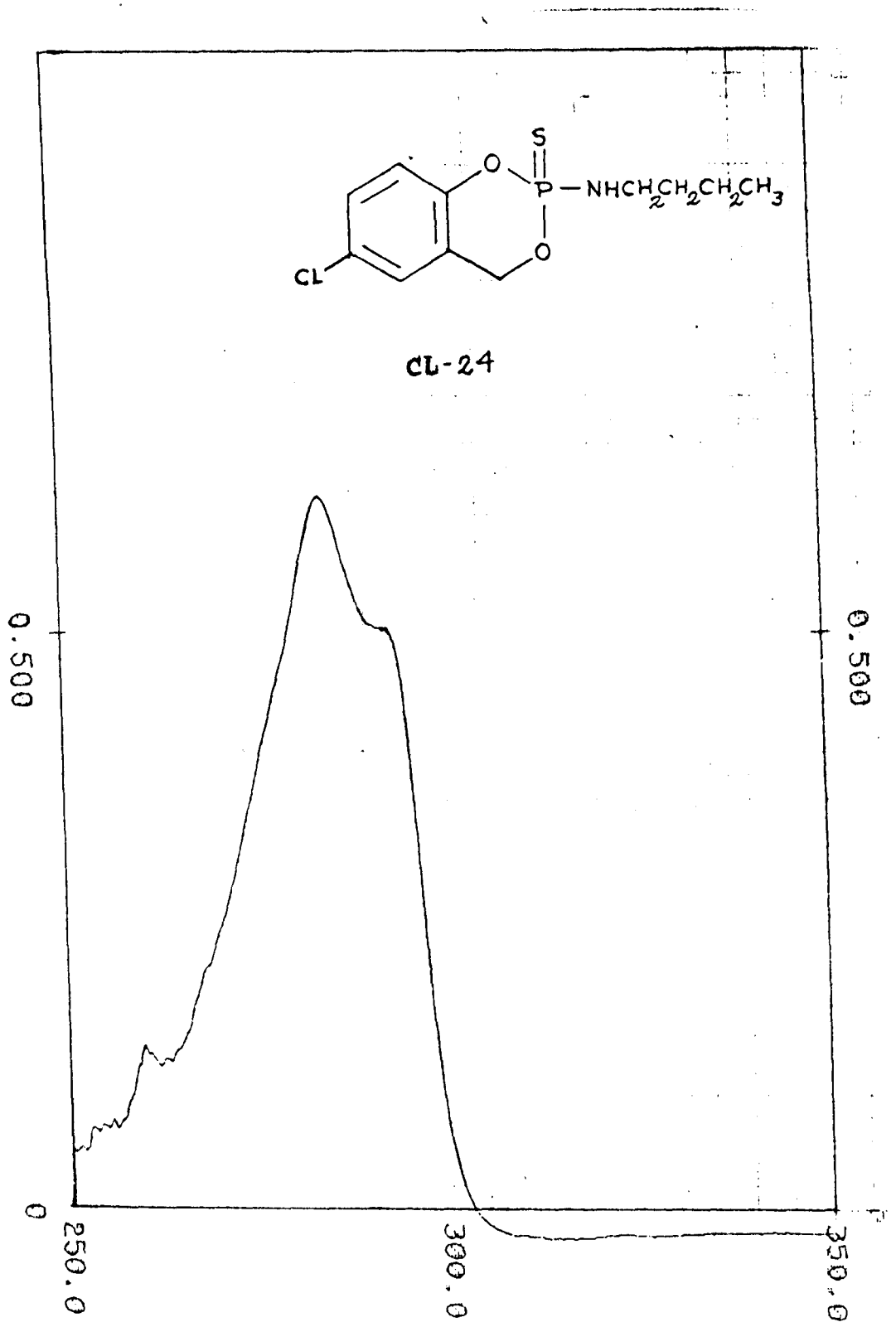


Fig.17 UV spectrum of N-butylamido-6-chloro-4H-1,3,2-benzodioxaphosphorin-2-sulphide (Cl-24)

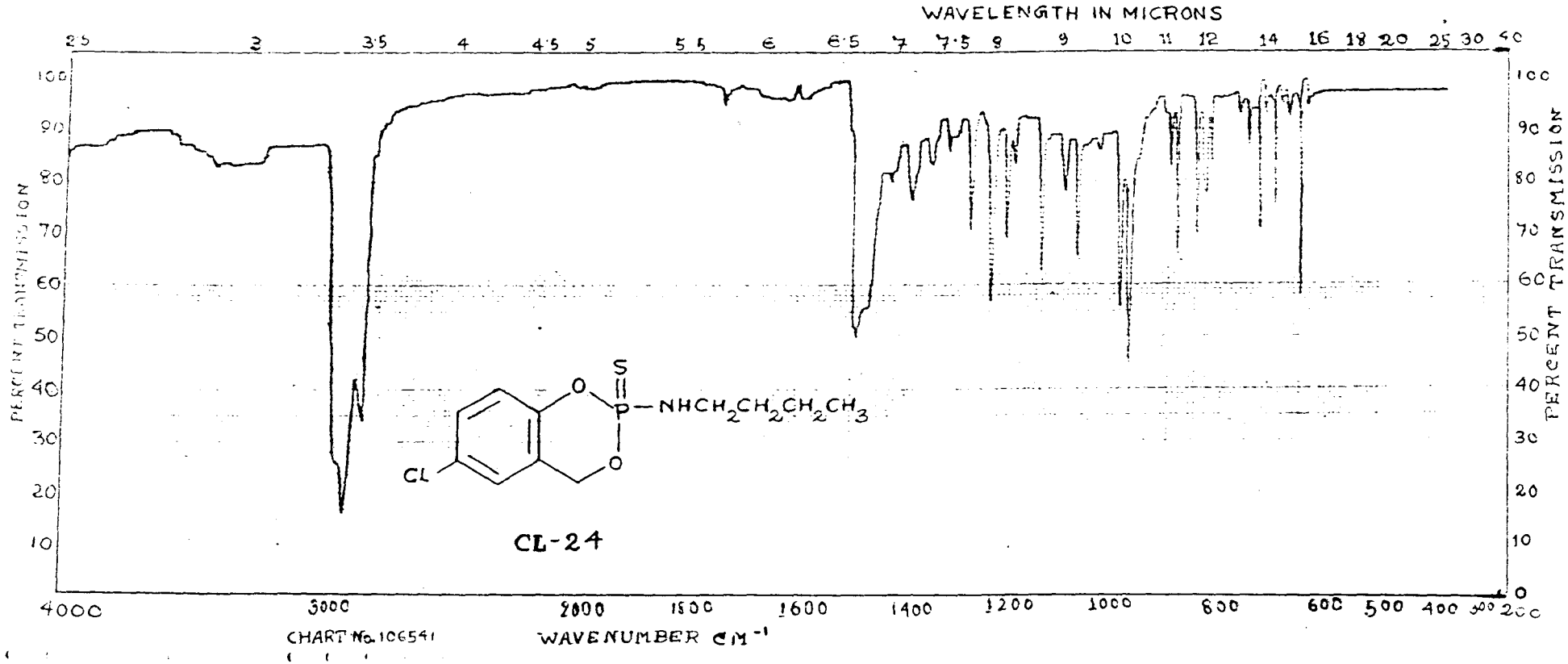


Fig.18 IR spectrum of N-butylamido-6-chloro-4H-1,3,2-benzodioxaphosphorin 2-sulphide (CL-24)

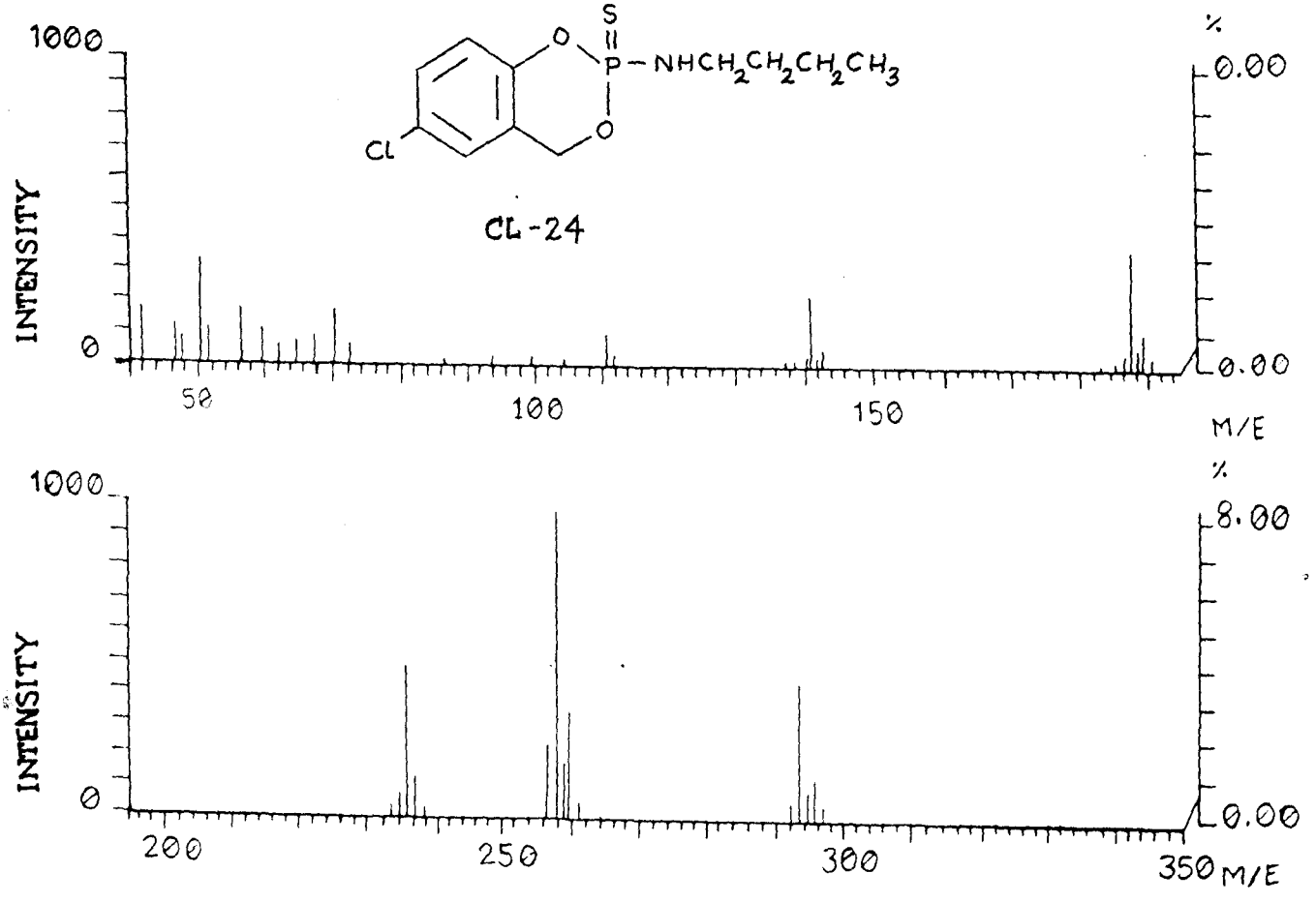


Fig.19 Mass spectrum of N-Butylamido-6-chloro-4H-1,3,2-benzodioxaphosphorin-2-sulphide (CL-24)

Mass (Fig. 19)

m/z	% RI
291.5 (M^+)	41.1
293.5 $(M + 2)^+$	12.7
258.5 (base peak)	100.0
260.5	31.2
187.5	38.5
189.5	11.8
140.5	25.2
142.5	8.1

(iv) 2-N,N-Diisobutylamido-6-bromo-4H-1,3,2-benzodioxaphosphorin-2-sulphide (BR-6):

UV (Fig. 20)

λ_{max}^{EtOH} = 278 nm (ϵ = 1666)

IR (Fig. 21)

1015 cm^{-1} (s)	P-O-C (alkyl)
1235-1260 cm^{-1} and 910 cm^{-1} (s)	P-O-C (aryl)
810 cm^{-1} (s)	P = S (I)
650 cm^{-1} (s)	P = S (II)
1040 cm^{-1} (s)	Ar-Br
730 cm^{-1} (s)	P-N str.

Mass (Fig. 22)

m/z		% RI
393	$(M + 2)^+$	15.0
391	(M^+)	15.0
358		20.0
348	(Base peak)	100.0
292		85.0
263		20.0
231		22.5
174		15.0
57		62.5
43		40.0

^{1}H NMR δ (Acetone - d_6/TMS) ppm (Fig. 23)

0.70-0.95 (12H, doublet, due to four $-CH_3$ group);

1.20-2.30 (2H, multiplet, due to two $-CH<$ group);

2.80-3.20 (4H, multiplet, due to $-N<^{CH_2-}_{CH_2-}$ Group);

$>CH_2$ group

4.90-5.60 (2H, eight line multiplet in the dioxaphorin ring);

6.80-7.45 (3H, multiplet due to aromatic hydrogen)

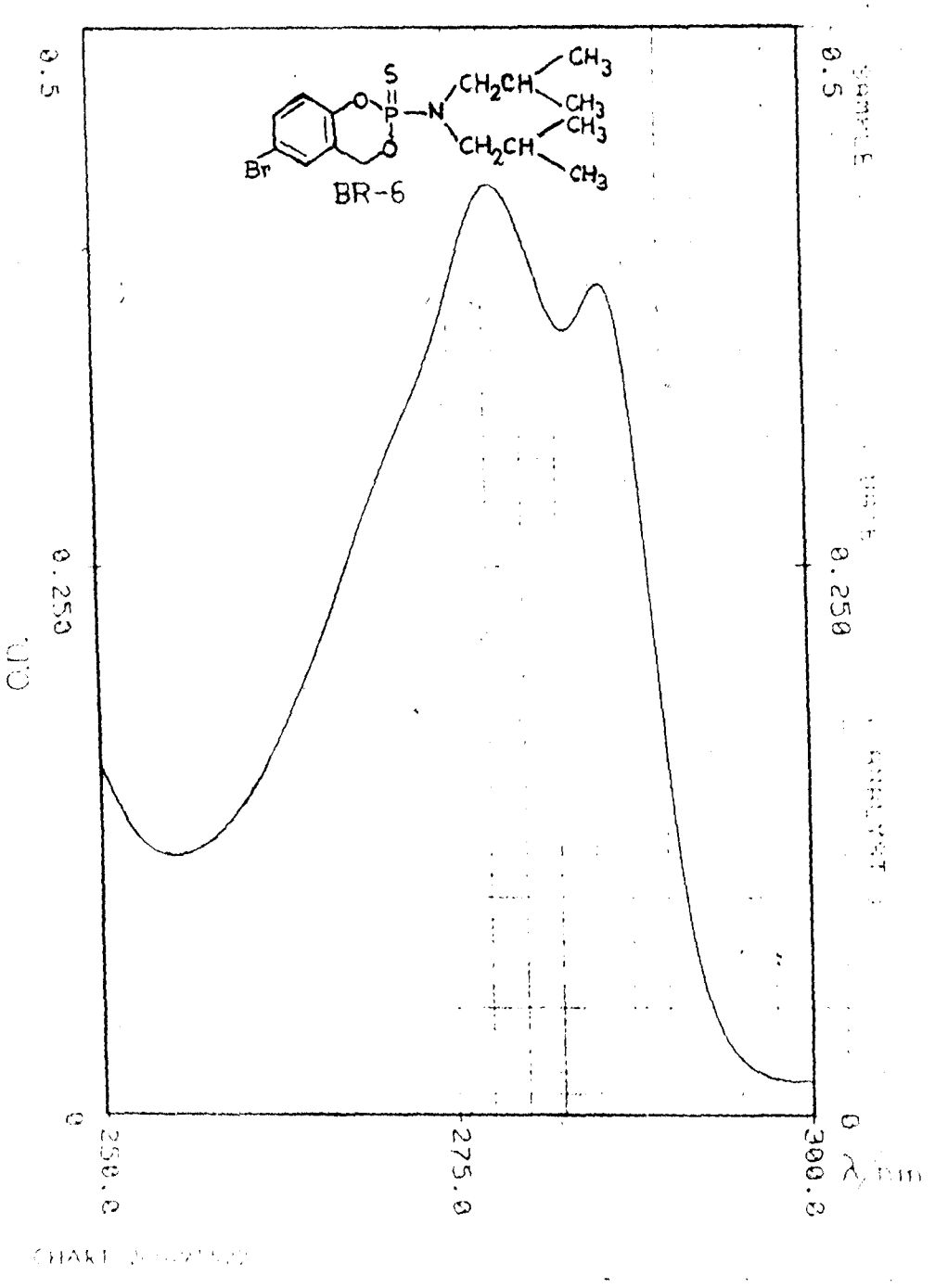


Fig.20 UV spectrum of 2-N,N-Diisobutylamido-6-bromo-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BR-6).

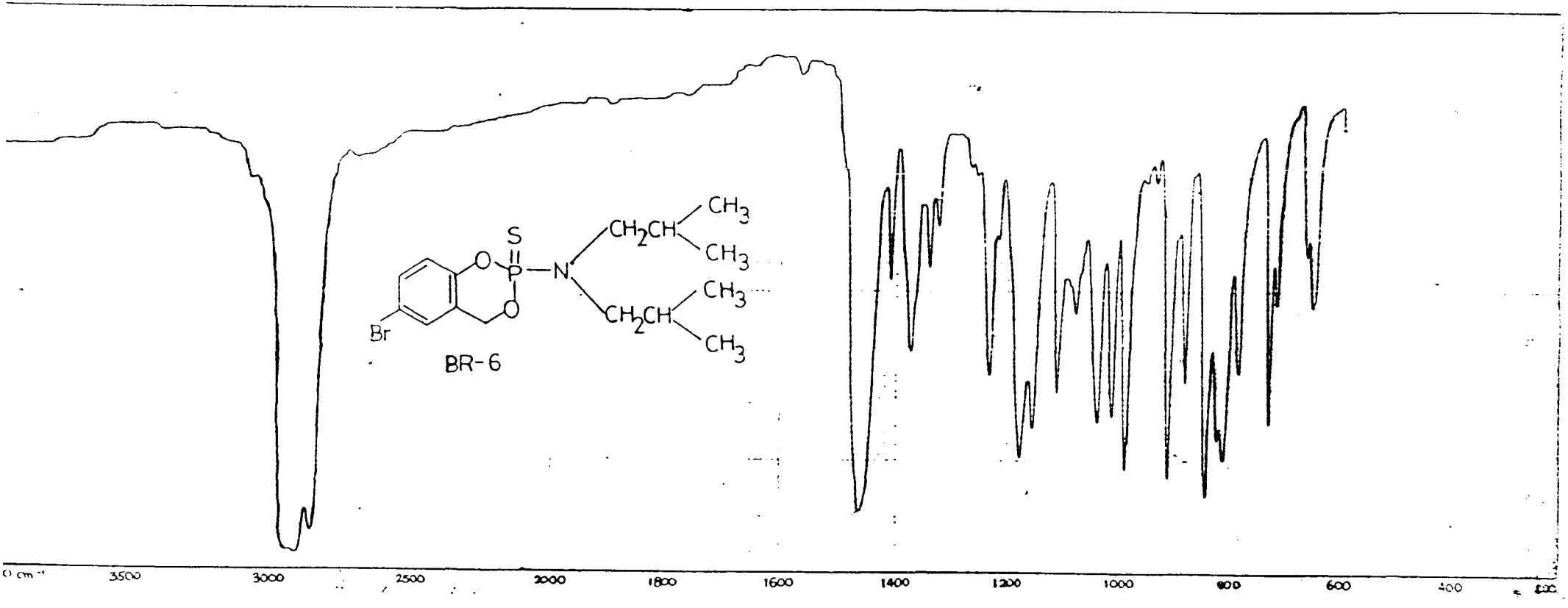


Fig. 21 IR spectrum of 2-N,N-Diisobutylamido-6-bromo-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BR-6)

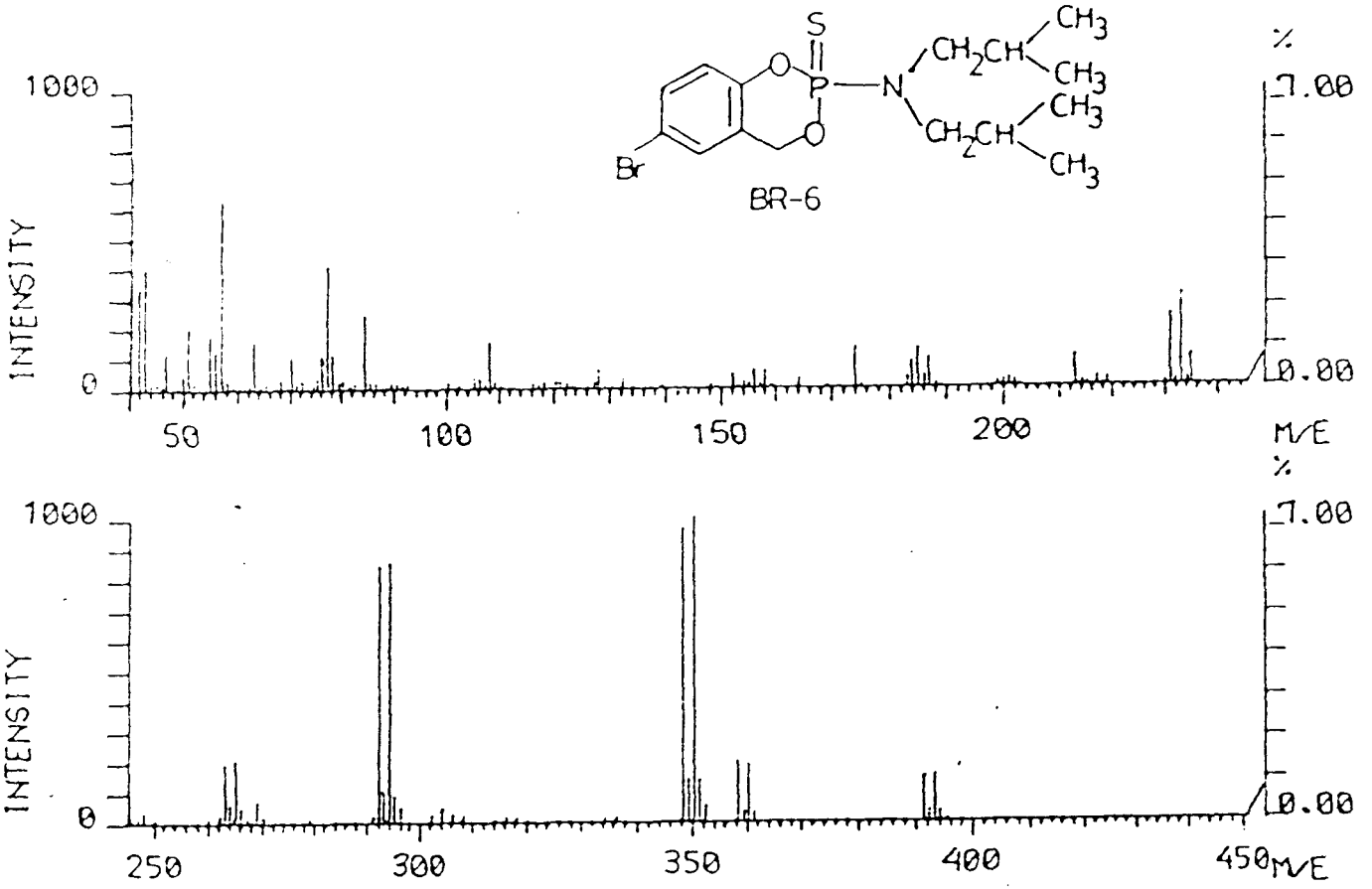


Fig.22 Mass spectrum of 2-N,N-Diisobutylamido-6-bromo-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BR-6).

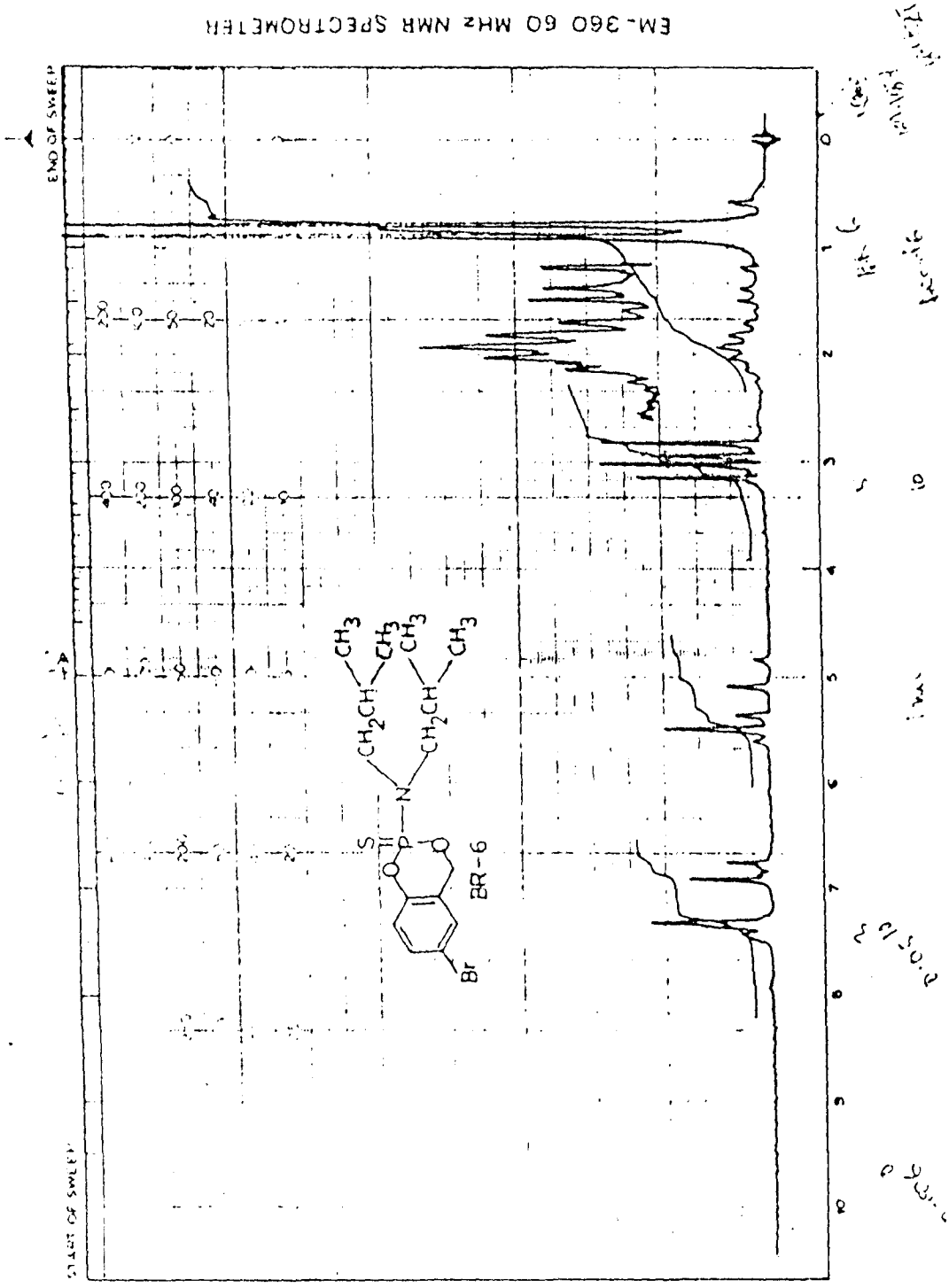


Fig 23. ^1H NMR spectrum of 2-N,N-Diisobutylamido-6-bromo-4H-1,3,2-benzodioxaphospnorin 2-sulpnide (BR-6).

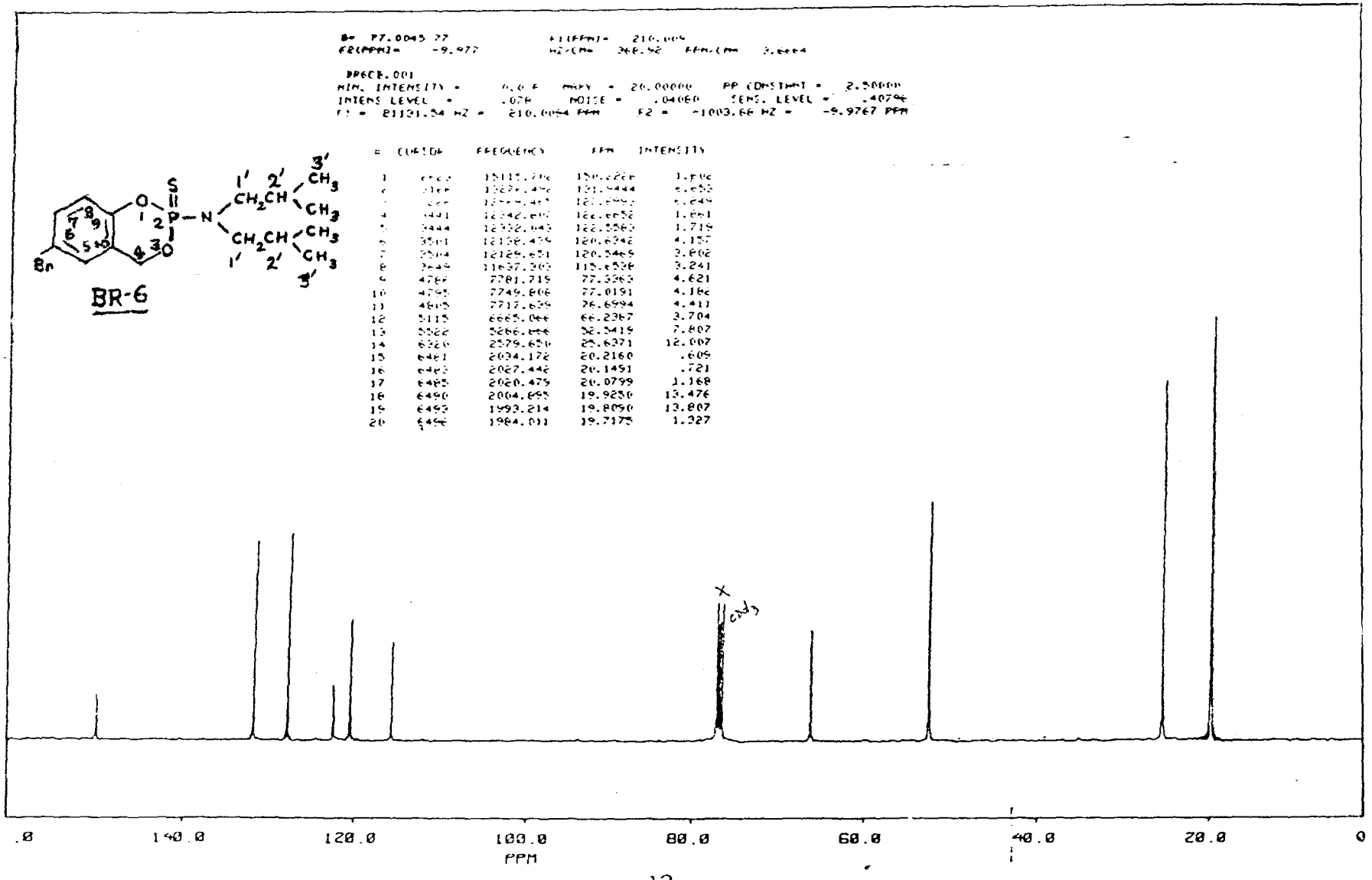


Fig.24 . 100 MHz proton noise decoupied ^{13}C spectrum of 2-N,N-Diisobutylamido-6-bromo-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BR-6)

^{13}C NMR ($CDCl_3$/TMS) ppm (Fig. 24):

^{13}C atom	Values (ppm)	$^{n}J_{AB}$*	Coupling constant (Magnitude in H_z)
C_3'	19.92 19.80	$^{4}J_{P-N-C_1'-C_2'-C_3'}$	11.68
C_2'	25.64		
C_1'	52.54		
C_4	66.24		
C_5	115.65		
C_8	120.63 120.55	$^{3}J_{P-O-C_9-C_8}$	8.78
C_{10}	122.67 122.56	$^{3}J_{P-O-C_9-C_{10}}$	10.76
C_7	127.89		
C_6	131.94		
C_9	150.22		

* $^{n}J_{AB}$ is used to represent a coupling over n bonds between nuclei A and B.

(v) 2-N,N-Dibutylamido-6-bromo-4H-1,3,2-benzodioxaphosphorin-2-sulphide (BR-10):

UV (Fig. 25)

λ_{max}^{EtOH} = 278 nm (ϵ = 1162)

IR (Fig. 26)

1020 cm^{-1} (s)	P-O-C (alkyl)
1250-1260 cm^{-1} (s) and	
900 cm^{-1} (s)	P-O-C (aryl)
810 cm^{-1} (s)	P = S (I)
650 cm^{-1} (s)	P = S (II)
1050 cm^{-1} (s)	Ar-Br
730 cm^{-1} (s)	P-N str.

Mass (Fig. 27)

m/z	% RI
393 $(M + 2)^+$	65.00
391 (M^+)	57.50
358 (Base peak)	100.00
348	22.50
305	22.50
292	16.25
263	10.00
231	23.75
174	50.00
57	17.50

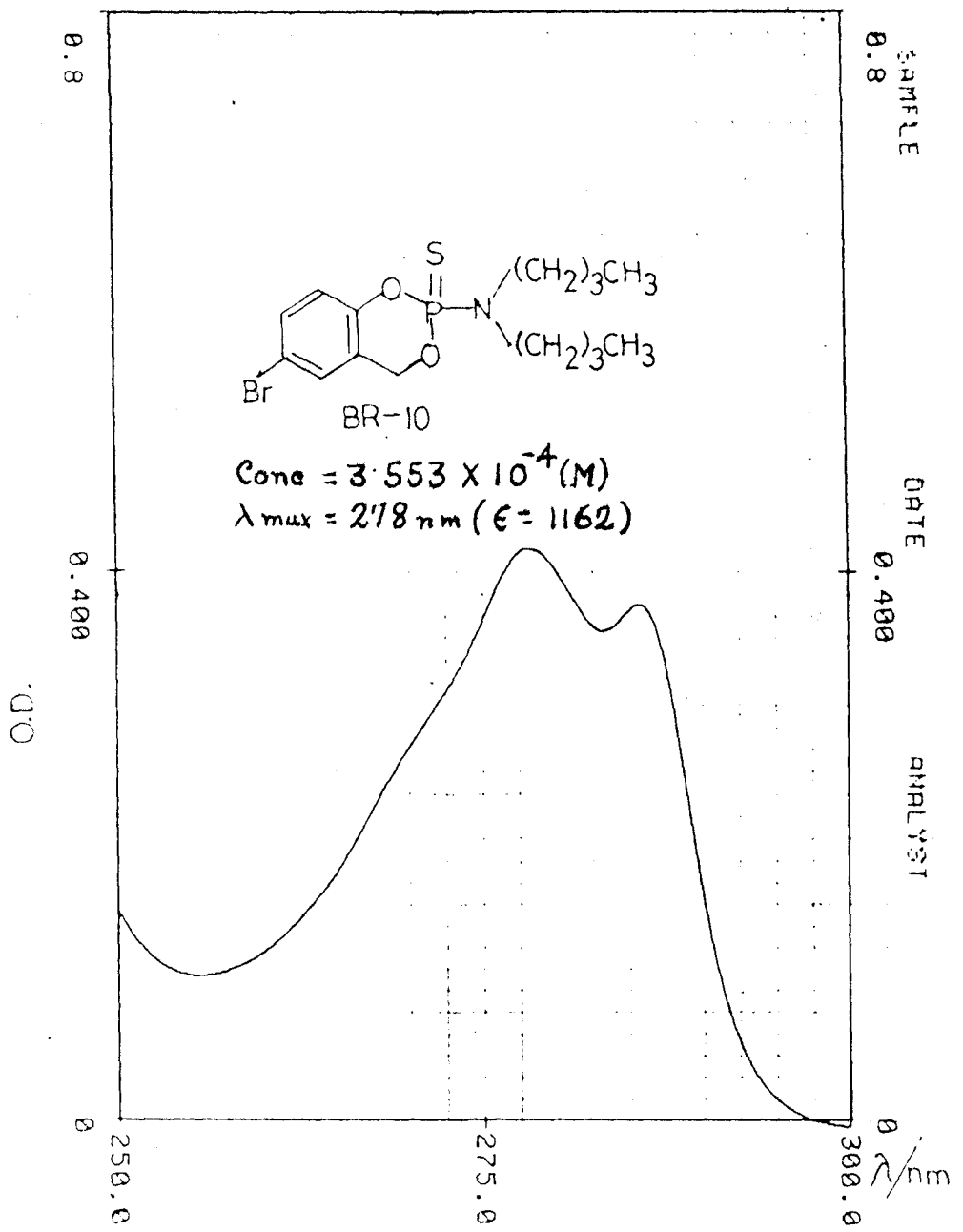


Fig.25 UV spectrum of 2-N,N-Dibutylamido-6-bromo-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BR-10)

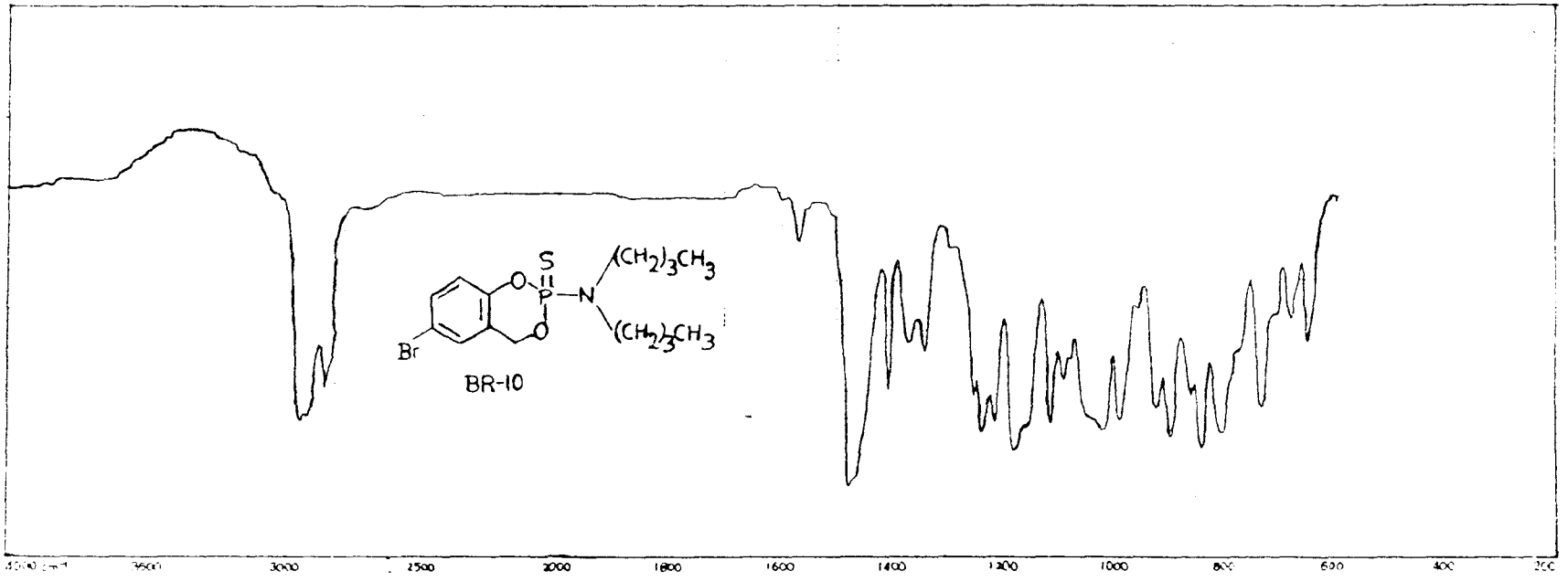


Fig.26 IR spectrum of 2-N,N-Dibutylamido-6-bromo-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BR-10)

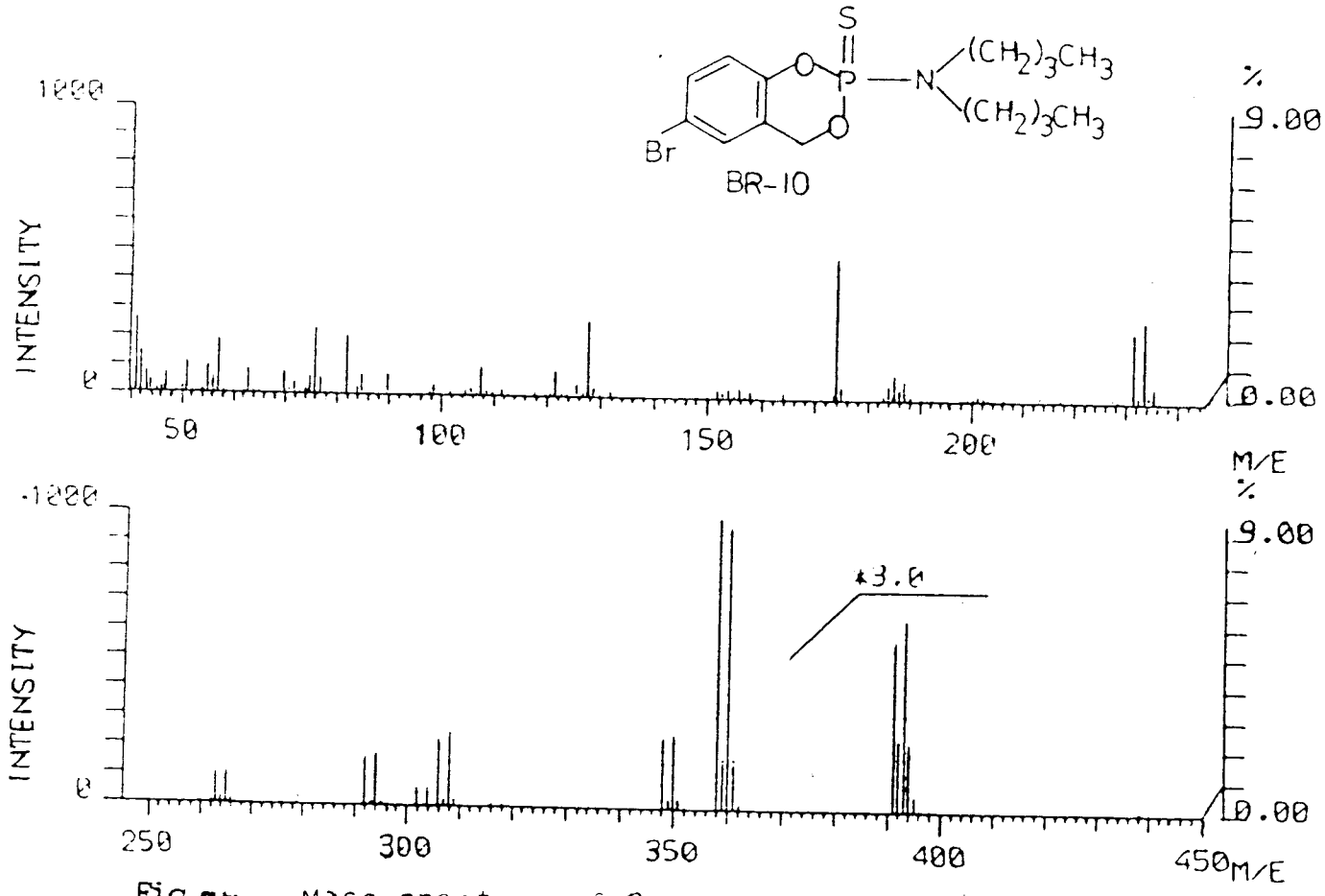


Fig. 27 Mass spectrum of 2-N,N-Dibutylamido-6-bromo-4H-1,3,2-benzodioxapnospnorin 2-sulphide (BR-10).

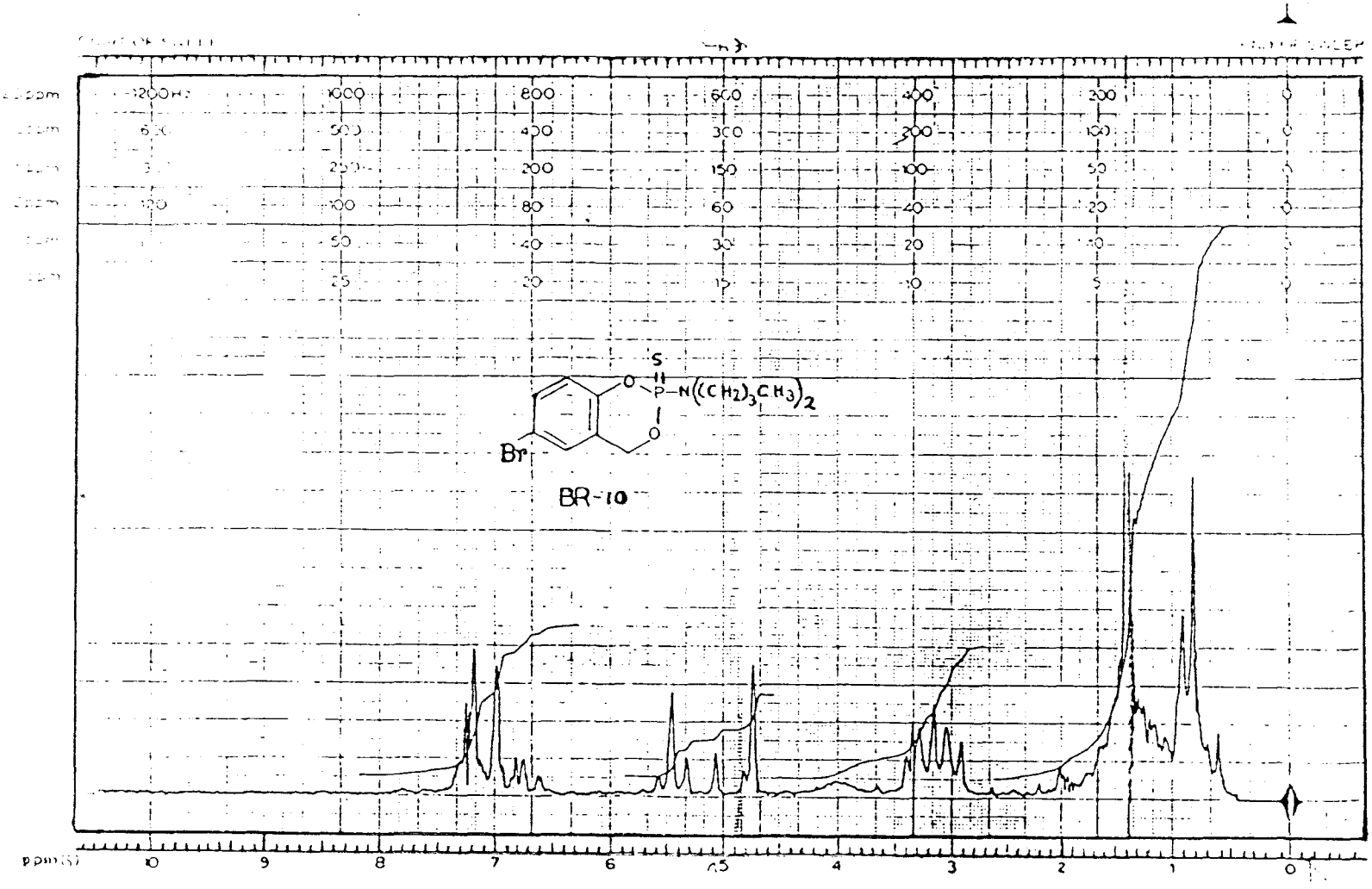


Fig.28 : ^{1}H NMR spectrum of 2-N,N-Dibutylamido-6-bromo-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BR-10).

^{1}H NMR (Acetone - d_6/TMS) ppm (Fig. 28)

0.80-0.90	(6H, triplet, due to two $-CH_3$ groups);
1.20-2.10	(8H, multiplet, due to two $-CH_2-CH_2-$ group);
2.91-3.40	(4H, multiplet, $-N(CH_2)_2$ group;
4.73-5.56	(2H, multiplet, $-CH_2-O-P$ group);
6.80-7.46	(3H, multiplet, aromatic hydrogen)

(vi) N-Butylamido-6-bromo-4H-1,3,2-benzodioxaphosphorin-2-sulphide (BR-24):

UV (Fig. 29)

λ_{max}^{EtOH} = 278 nm (ϵ = 1008)

IR (Fig. 30)

1025 cm^{-1}	(s)	P-O-C (alkyl)
1240 cm^{-1}	(s) and	
900 cm^{-1}	(s)	P-O-C (aryl)
805 cm^{-1}	(s)	P = S (I)
665 cm^{-1}	(s)	P = S (II)
735 cm^{-1}	(s)	P-N str.
3280 cm^{-1}	(s)	N-H str.

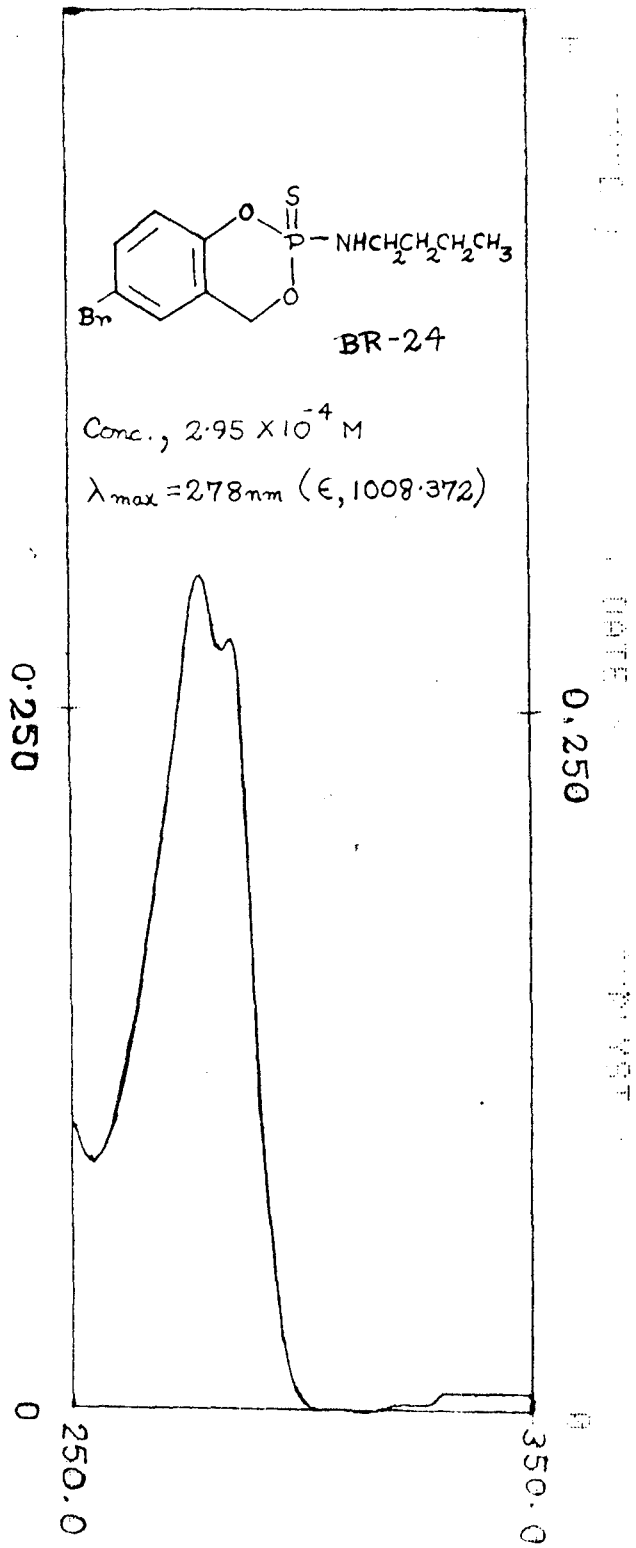


Fig.29 UV spectrum of 2-N-butylamido-6-bromo-4H-1,3,2-benzodioxaphosphor
2-sulphide (BR-24)

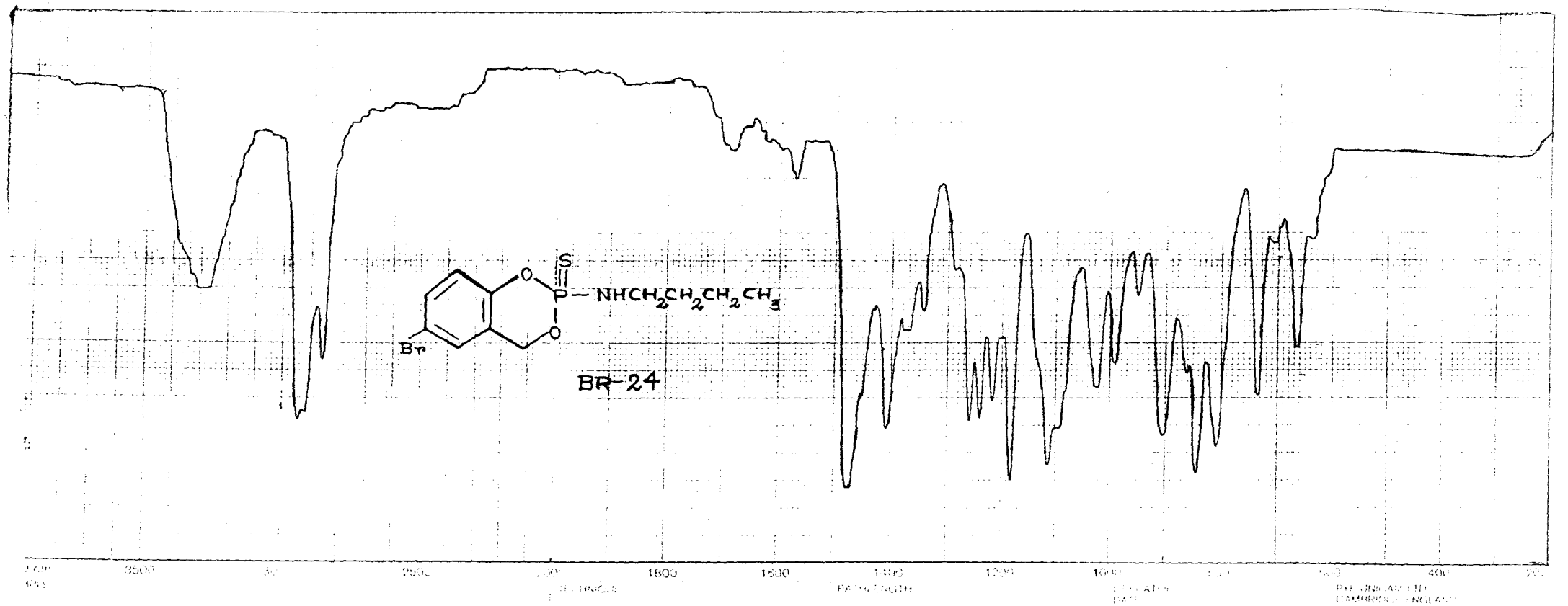


Fig.30 IR spectrum of 2-N-butylamido-6-bromo-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BR-24)

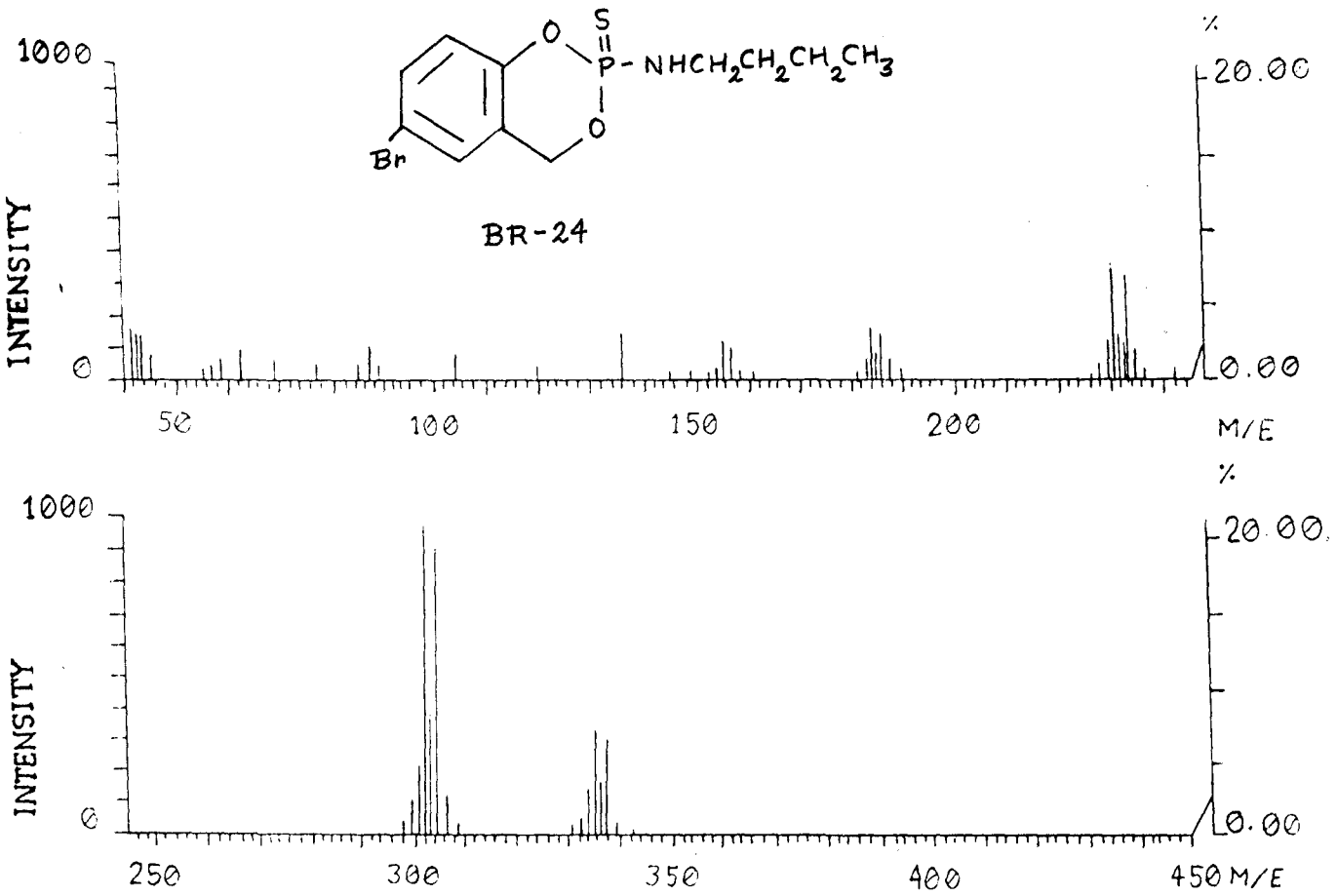


Fig. 31 Mass spectrum of N-Butylamido-6-bromo-4H-1,3,2-benzodioxaphosphorin-2-sulphide (BR-24).

Mass (Fig. 31)

m/z	% RI
335 (M^+)	35.5
337 $(M + 2)^+$	34.8
302 (base peak)	100.0
304	99.5
231	31.1
233	30.5
184	21.5
186	20.9
156	15.3
158	14.7

(vii) 2-Hexylamido-6-bromo-4H-1,3,2-benzodioxaphosphorin-2-sulphide (BR-27):

UV (Fig. 32)

λ_{max}^{EtOH} = 283 nm (ϵ = 2923)

IR (Fig. 33)

1070 cm^{-1} (s)	P-O-C (alkyl)
1240 cm^{-1} (s) and	
890 cm^{-1} (s)	P-O-C (aryl)
810 cm^{-1} (s)	P = S (I)
660 cm^{-1} (m)	P = S (II)
3280 cm^{-1} (s)	N-H str.
1620 cm^{-1} (w) and	Two components of the sbustituted
1565 cm^{-1} (w)	benzene ring a quadrant str.
735 cm^{-1} (s)	P-N str.

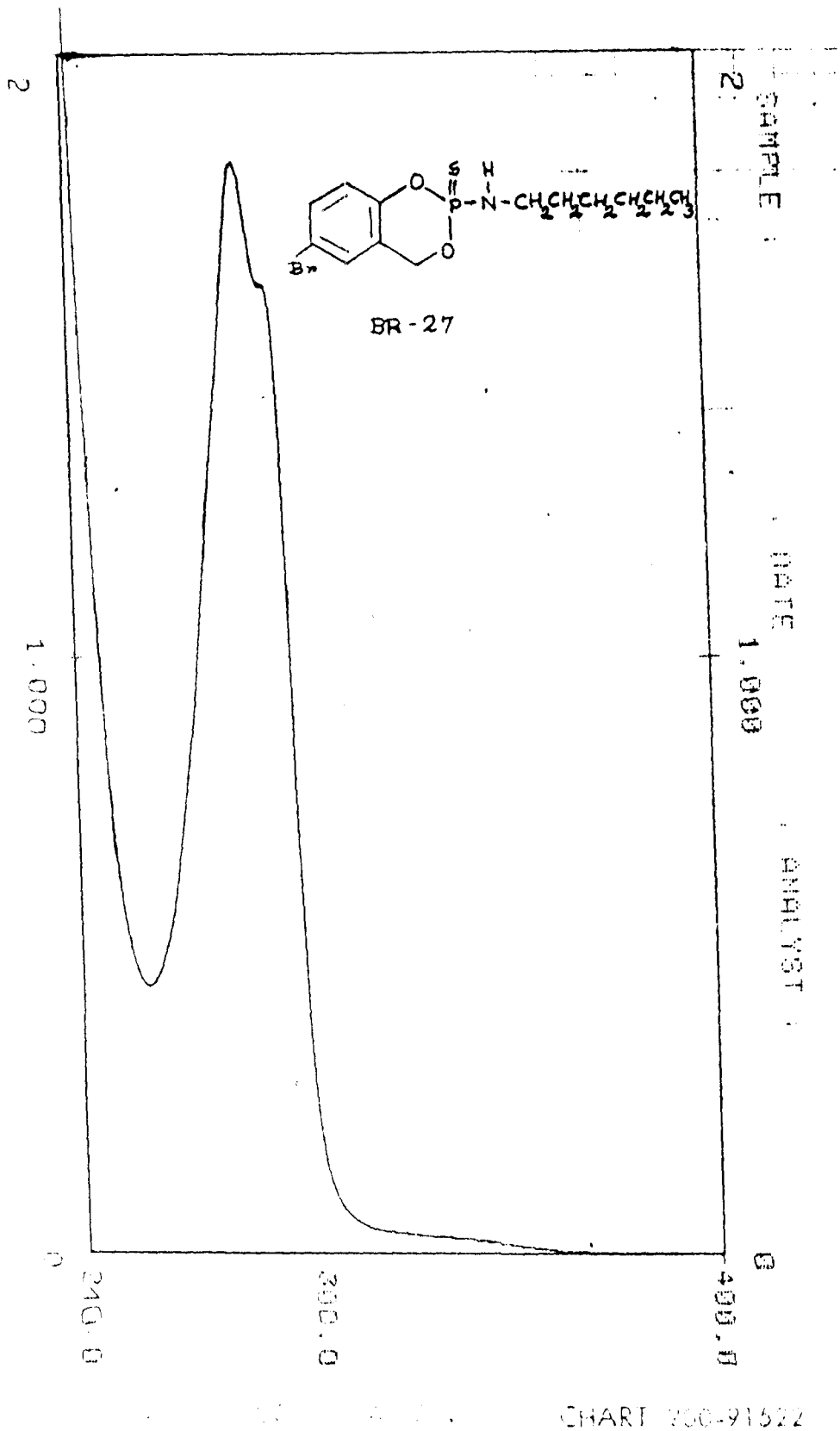


Fig. 32 UV spectrum of 2-Hexylamido-6-bromo-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BR-27)

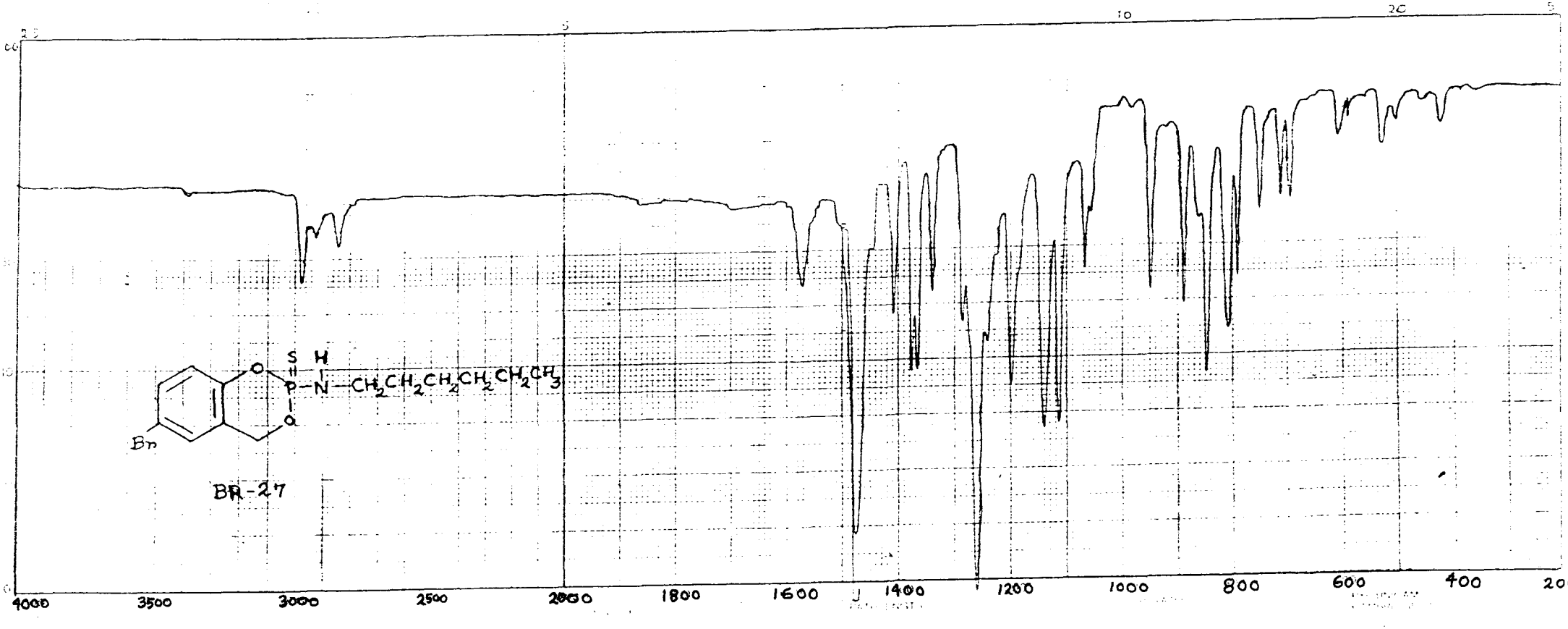


Fig.33 IR spectrum of 2- Hexylamido-6-chloro-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BR-27)

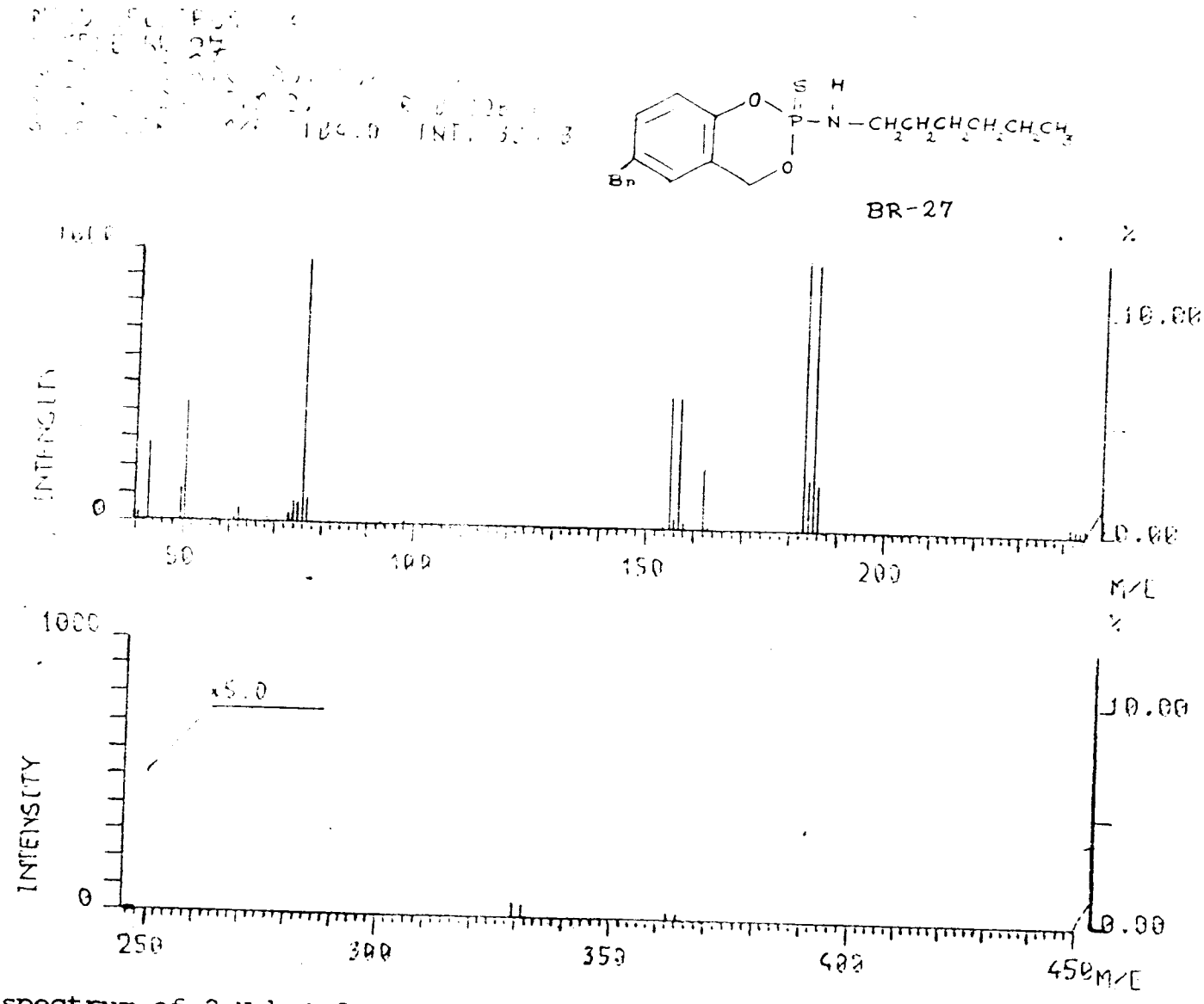


Fig. 34 Mass spectrum of 2-N-butylamido-6-chloro-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BR-27)

Mass (Fig. 34)

m/z		% RI
363	(M^+)	1.00
365	$(M + 2)^+$	1.00
330		2.00
332		0.50
184	(base peak)	100.00
186		99.00
156		49.00
158		48.70
77		97.00

(viii) 2-(β-methoxy)ethoxy-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-sulphide (BD-1):

UV (Fig. 35)

λ_{max}^{EtOH} = 280 nm (ϵ = 9352)

IR (Fig. 36)

1020 cm^{-1} (s)	P-O-C (alkyl);
1050 cm^{-1} (s)	P-O-C (alkyl);
1190 cm^{-1} (s)	P-O-C (aryl);
920 cm^{-1} (s)	P-O-C (aryl);
1515 cm^{-1} (s)	asym. str. of nitro group;
1340 cm^{-1} (vs)	sym. str. of nitro group;
800 cm^{-1} (s)	P = S (I);
1615 cm^{-1} (w) and	benzene ring "quadrant stretching"
1580 cm^{-1} (m)	C = C vibrations.

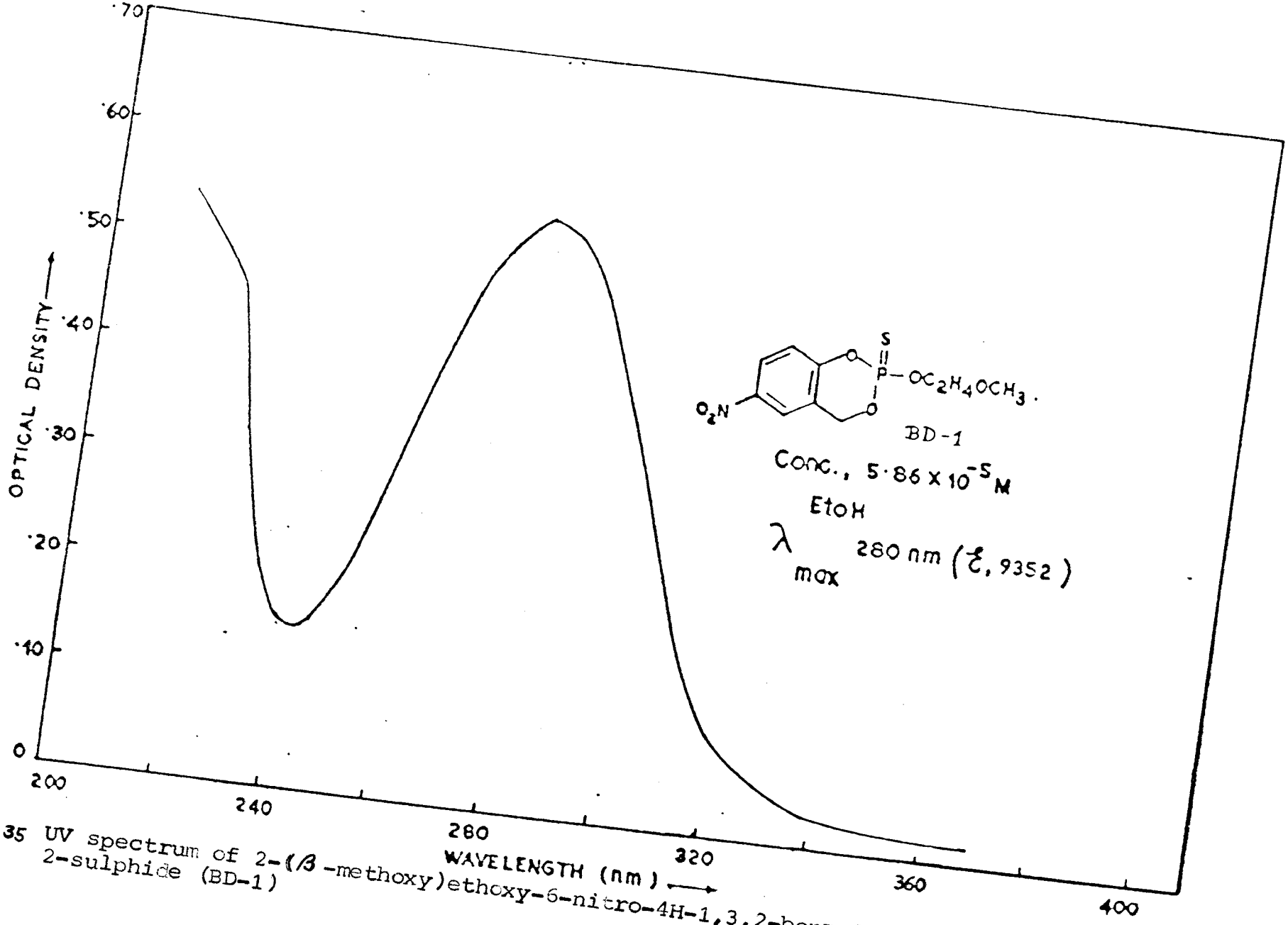


Fig.35 UV spectrum of 2-(β-methoxy)ethoxy-6-nitro-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BD-1)

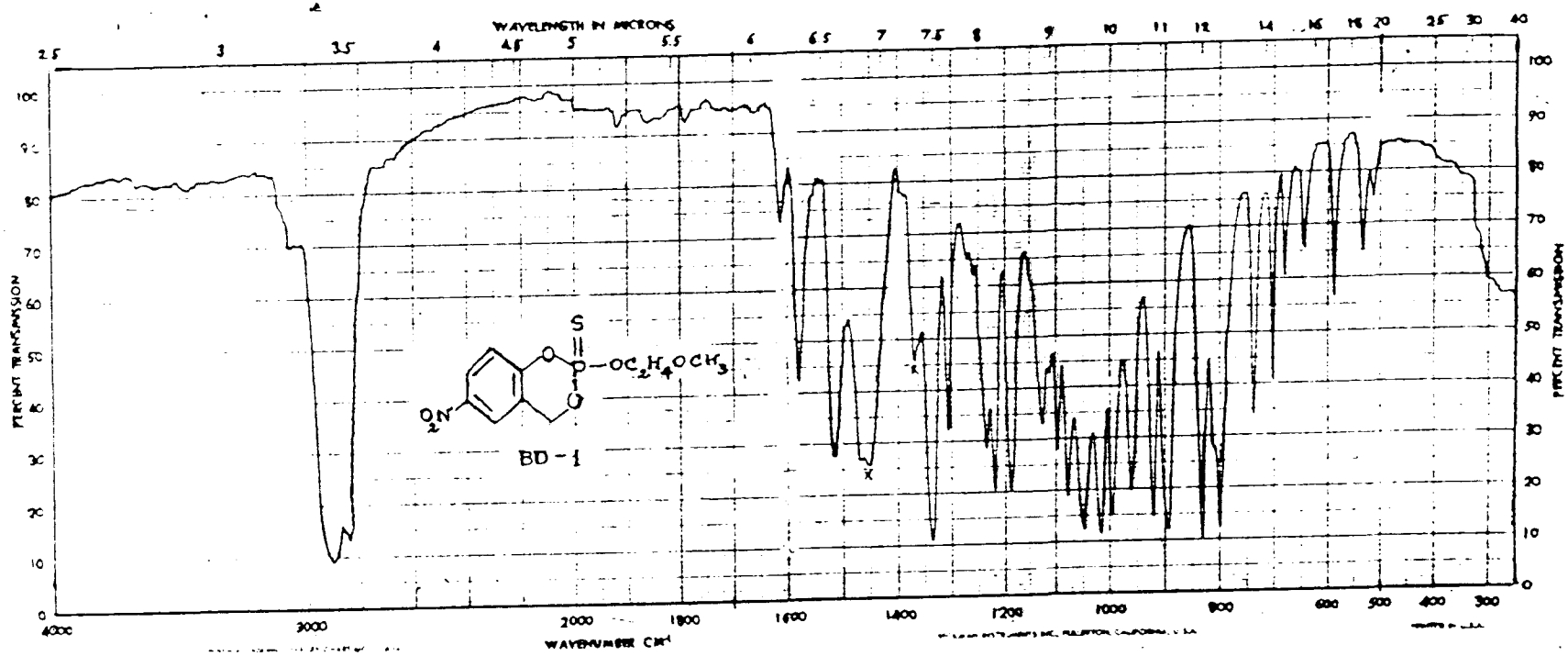


Fig.36 IR spectrum of 2(β-methoxy)ethoxy-5-nitro-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BD-1)

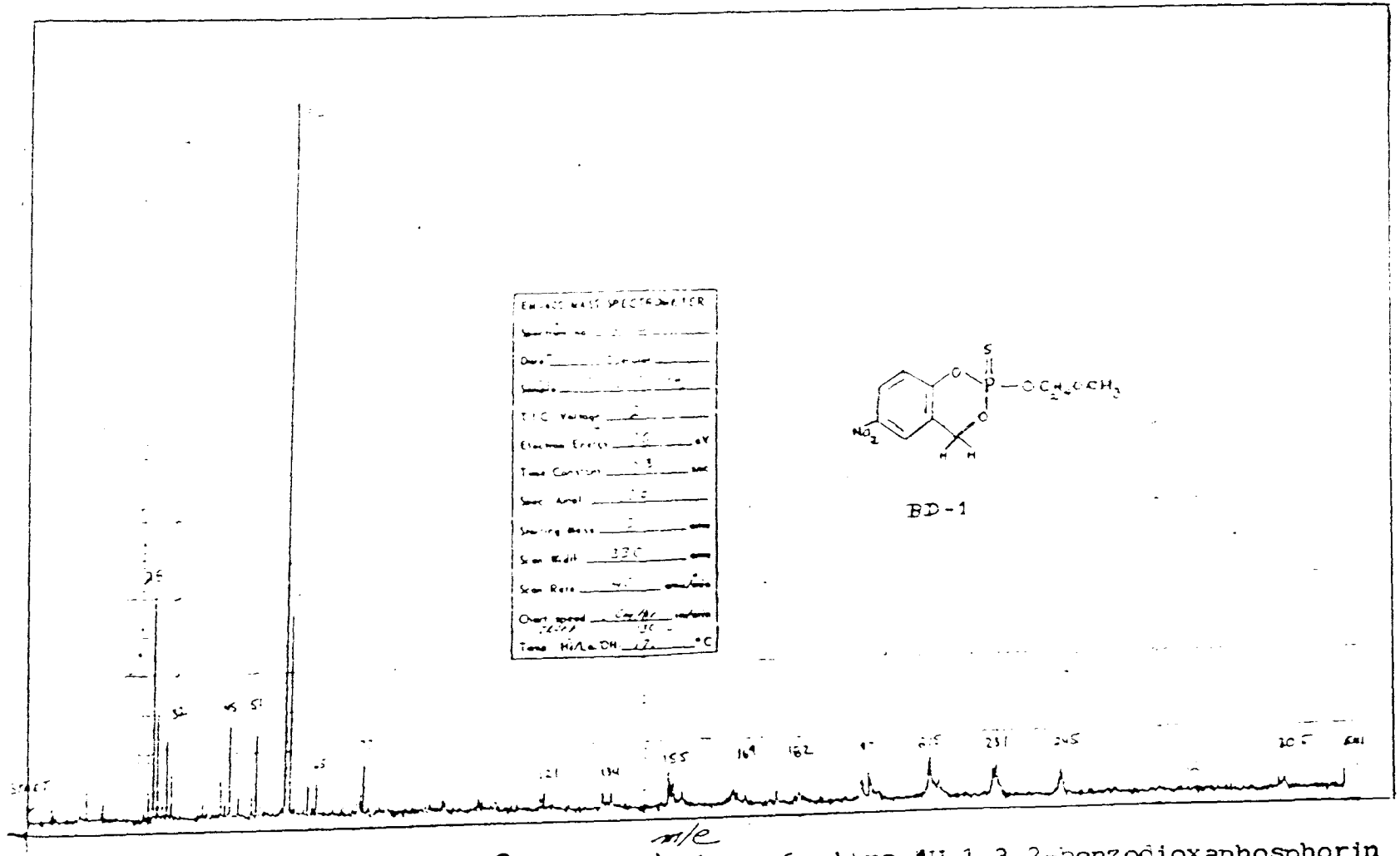


Fig. 37 Mass spectrum of 2(β-methoxy)ethoxy-6-nitro-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BD-1)

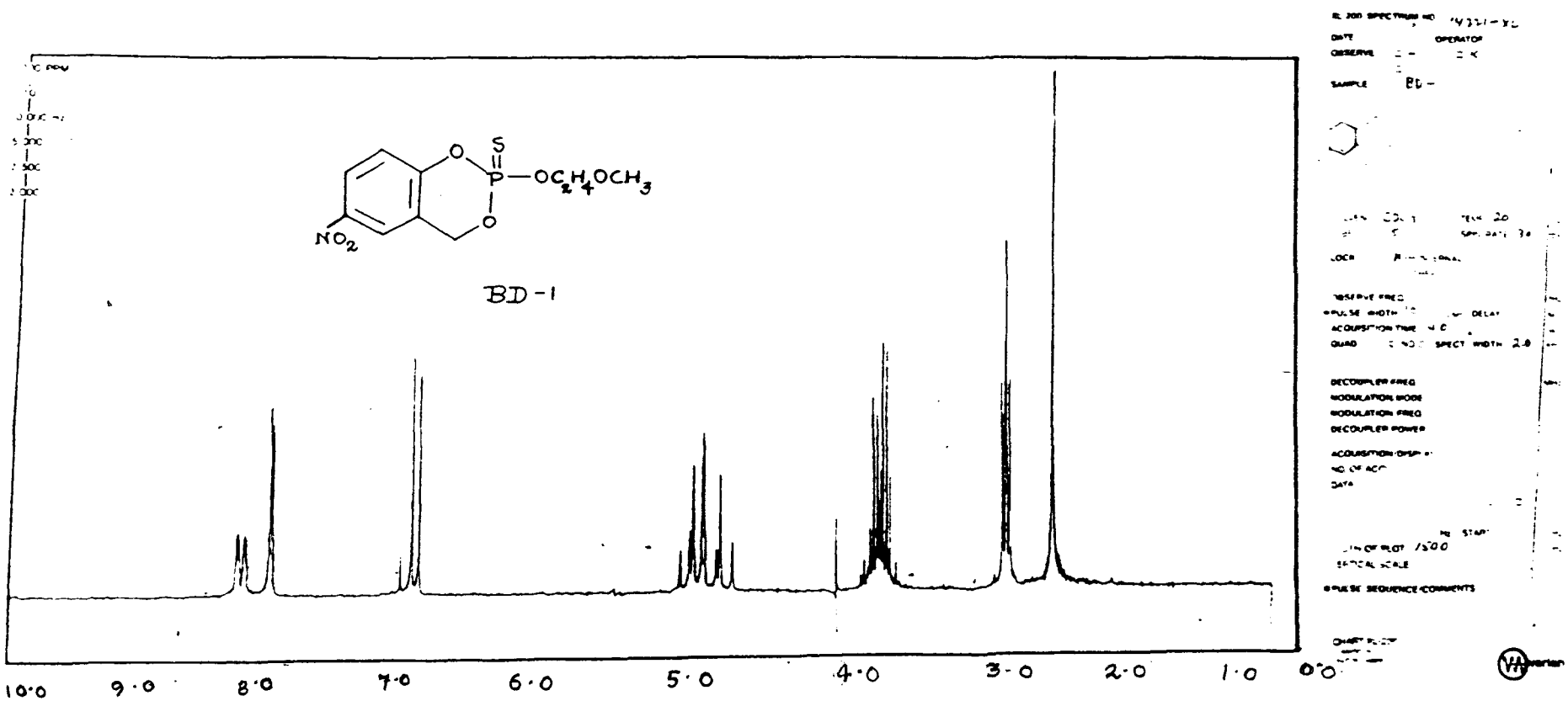


Fig. 38 ^{1}H NMR spectrum of 2(β-methoxy)ethoxy-6-nitro-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BD-1)

Mass (Fig. 37)

m/z		% RI
305	(M^+)	1
59		26
58	(base peak)	100
51		10
45		12

^{1}H NMR δ ($CDCl_3$) ppm (Fig. 38)

3.35	(3H, singlet, $-OCH_3$);
3.6	(2H, triplet, $-CH_2OCH_3$ group, slight further splittings due to phosphorus are also observed);
4.3	(2H, multiplet, $P-O-CH_2-CH_2-OCH_3$ group, splittings due to phosphorus are also observed);
5.4	(at 5.5 singlet and at 5.3 doublet, $-CH_2-$ group in the dioxaphosphorin ring);
7.1	(1H, doublet, one aromatic hydrogen meta to nitro group);
8.0	(1H, doublet, one aromatic hydrogen ortho to both nitro group and $-CH_2-$ group of the dioxaphosphorin ring);
8.2	(1H, doublet, remaining one aromatic hydrogen).

(ix) 2-Iso-proxpy-6-nitro-4H-1, 3,2-benzodioxaphosphorin 2-sulphide (BD-5):

UV (Fig. 39)

λ_{max}^{EtOH} = 280 nm (ϵ = 8092)

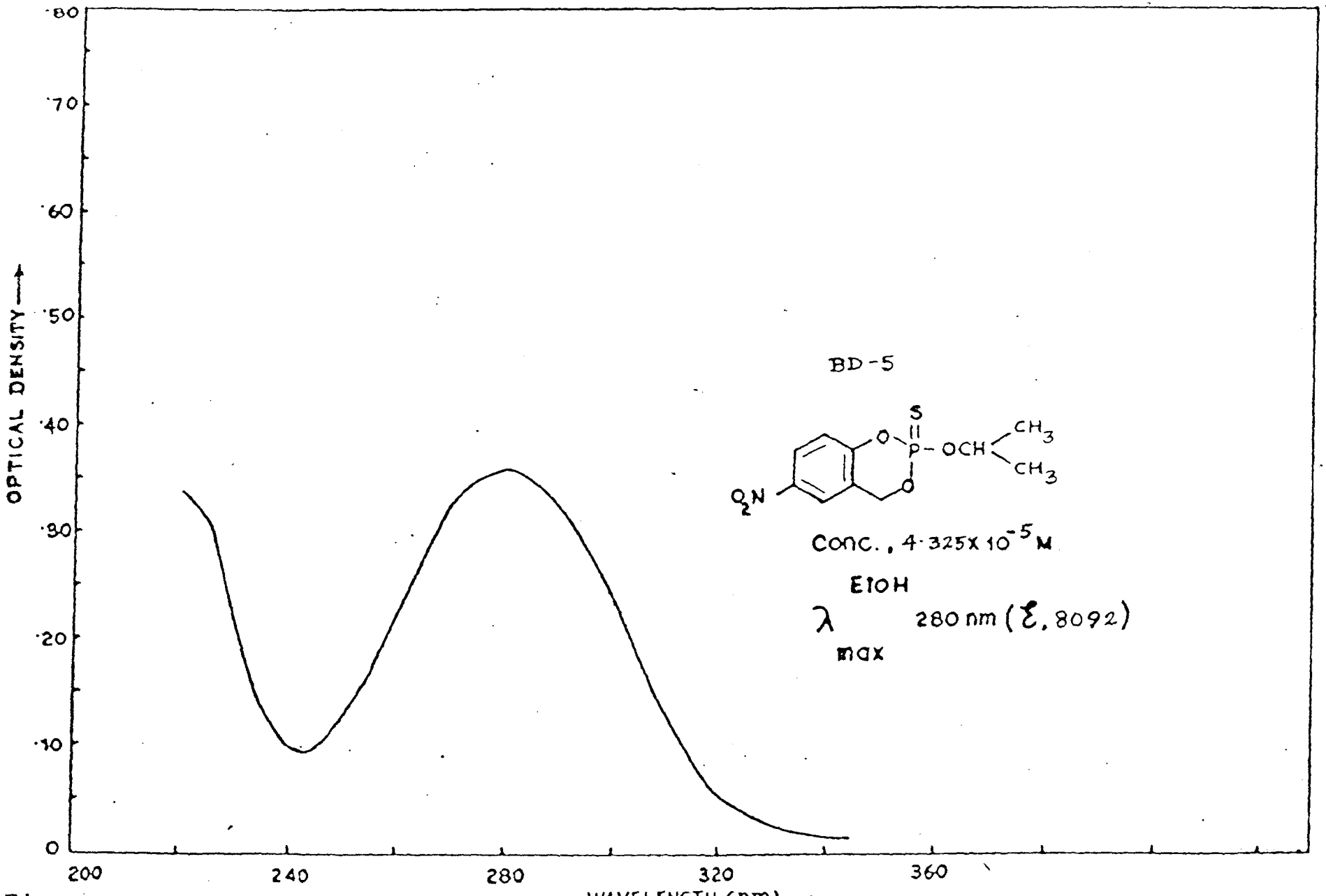


Fig.39 UV spectrum of 2-iso-propoxy-6-nitro-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BD-5)

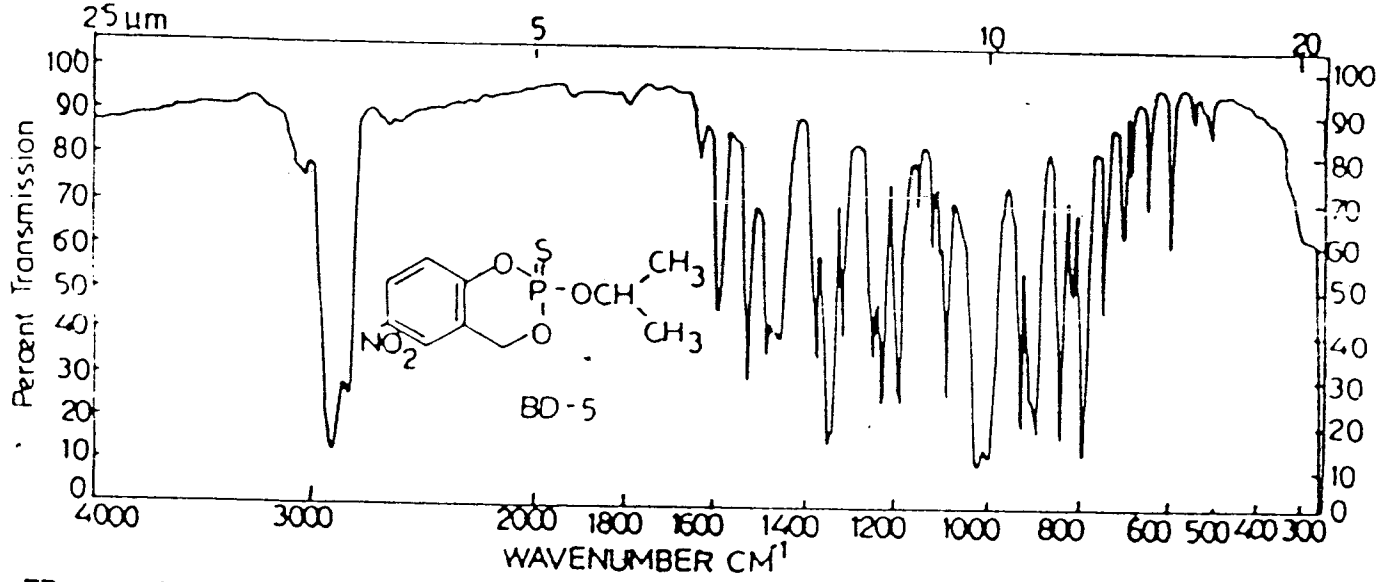


Fig.40 IR spectrum of 2-iso-propoxy-6-nitro-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BD-5)

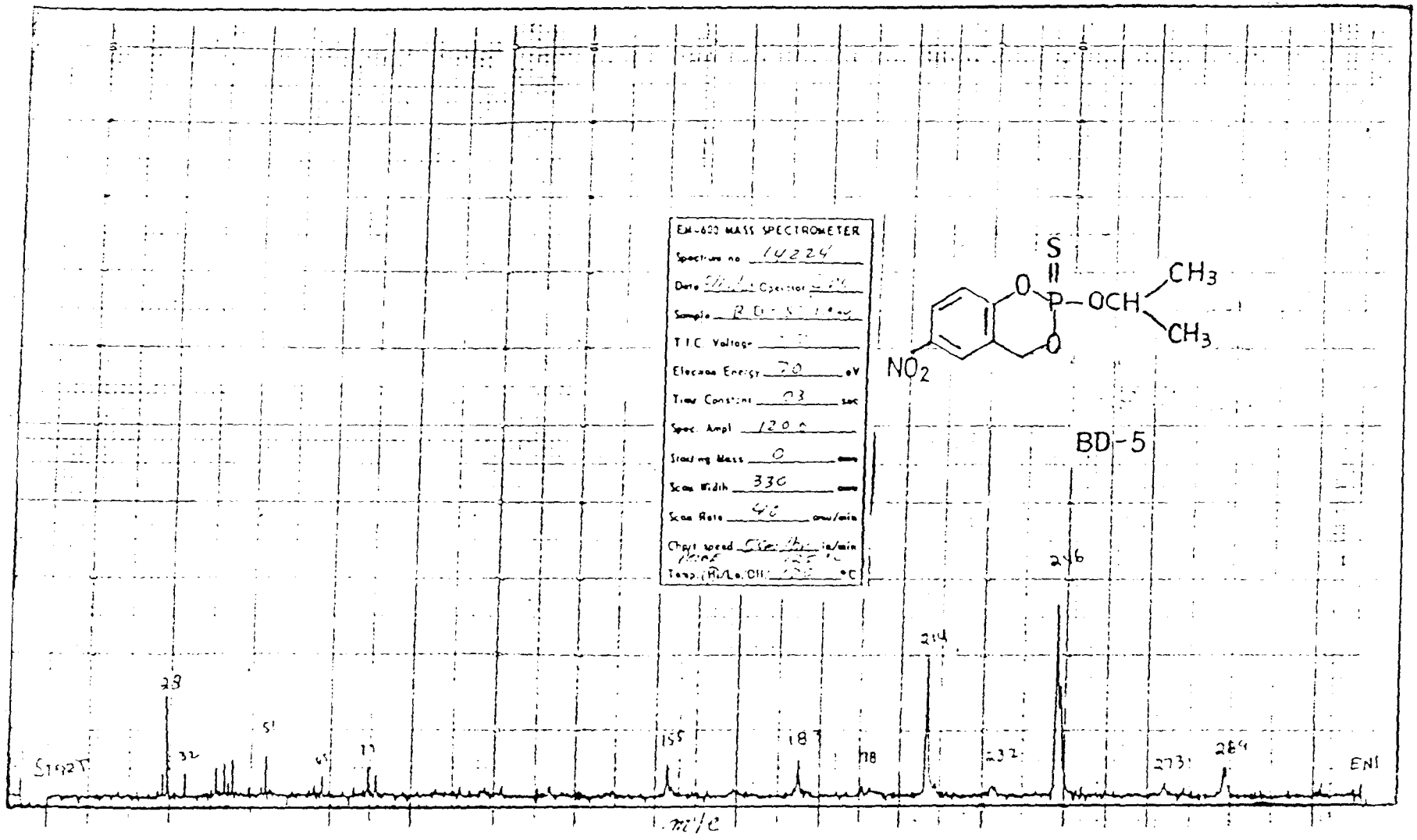


Fig.41 Mass spectrum of 2-iso-propoxy-6-nitro-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BD-5)

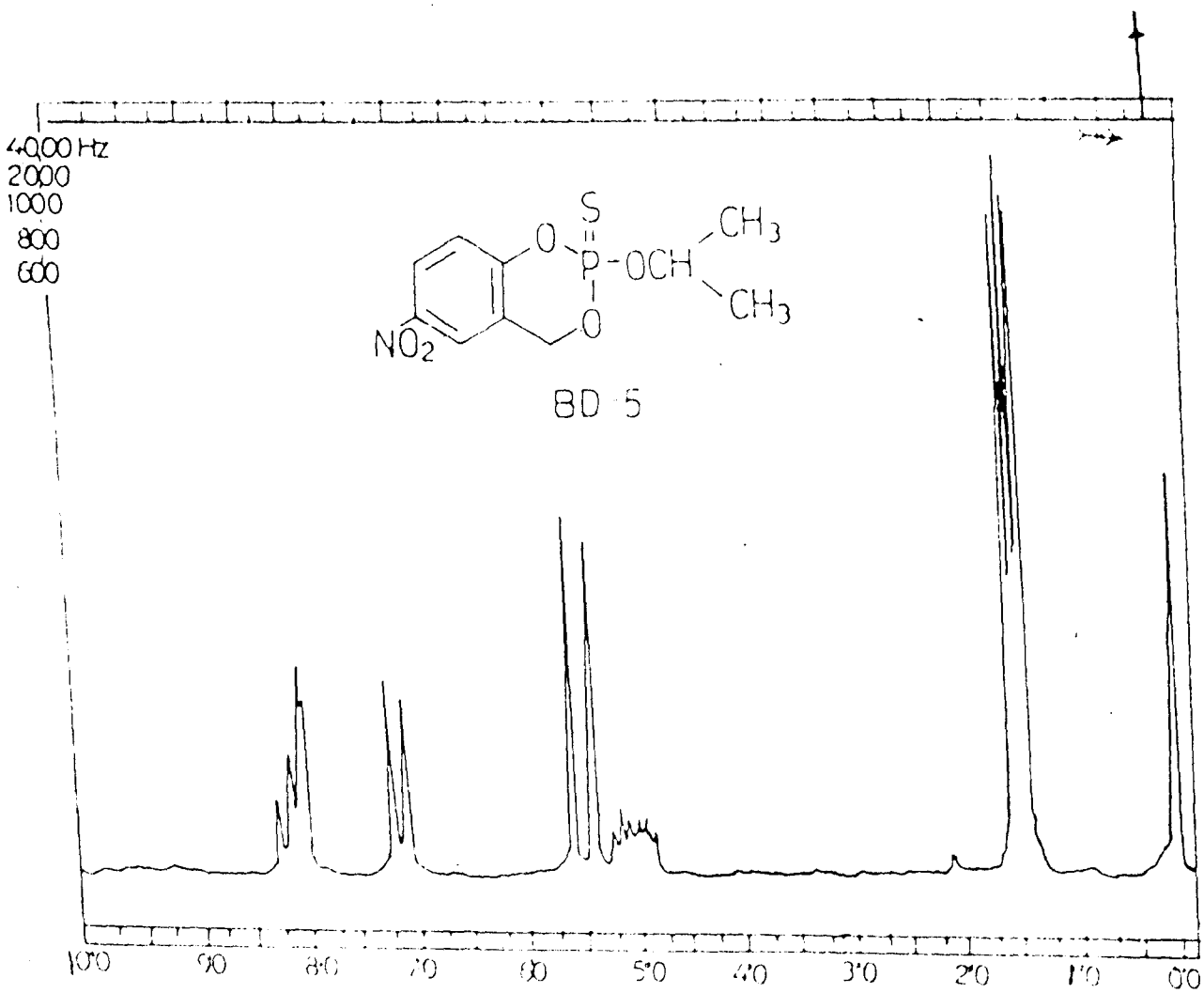


Fig.42 ^{1}H NMR spectrum of 2-iso-propoxy-6-nitro-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BD-5)

IR (Fig. 40)

1000 cm^{-1} (vs) and 1020 cm^{-1} (vs)	P-O-C (isopropyl);
1200 cm^{-1} (s) and 930 cm^{-1} (s)	P-O-C (aryl);
1526 cm^{-1} (s) and 1345 cm^{-1} (s)	asym. and sym. str. of nitro group respectively.
790 cm^{-1} (s)	P = S (I);
650 cm^{-1} (m)	P = S (II);
1620 cm^{-1} (w) and 1590 cm^{-1} (m)	benzene ring quadrant stretching C = C vibration.

Mass (Fig. 41)

M/z	% RI
289 (M^+)	16
247	56
246 (base peak)	100
214	72
198	4

^1H NMR δ ($CDCl_3$) ppm (Fig. 41)

1.4	(6H, quartet, two $-CH_3$ group);
4.9	(1H, multiplet, $-CH<$ group);
5.3	(1H, singlet, one hydrogen of $-CH_2$ group in dioxaphosphorin ring);
5.5	(1H, singlet, one hydrogen of $-CH_2-$ group in dioxaphosphorin ring);
7.1	(1H, doublet, J_{H-H} = 8.5 H_z, one aromatic hydrogen meta to nitro group);
8.0	(1H, doublet, one aromatic hydrogen ortho to both nitro group and $-CH_2-$ group of dioxaphosphorin ring);
8.2	(1H, doublet, J_{H-H} = 8.5 H_z, remaining one aromatic hydrogen).

(x) 2-Phenoxy-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-sulphide (BD - 9):

UV (Fig-42)

λ_{max}^{EtOH} = 277 nm (ϵ = 9755)

IR (Fig. 44)

1020 cm^{-1} (s)	P-O-C (alkyl);
950 cm^{-1} (s)	P-O-C (phenyl);
1185 cm^{-1} (s)	P-O-C (aryl);
905 cm^{-1} (s)	P-O-C (aryl);
1530 cm^{-1} (s)	asym. str. of nitro group;
1345 cm^{-1} (s)	sym. str. of nitro group;
785 cm^{-1} (s)	P = S (I)

Mass (Fig. 45)

m/z	% RI
323 (base peak)	100
293	60
250	99
235	75
214	99
198	82

^{1}H NMR δ ($CDCl_3$) ppm (Fig. 46)

5.5	(1H, doublet, one hydrogen of $-CH_2-$ group in dioxaphosphorin ring);
5.65	(1H, singlet, one hydrogen of $-CH_2-$ group in dioxaphosphorin ring).

7.25, 7.35, 8.1 and 8.2 are due to aromatic protons.

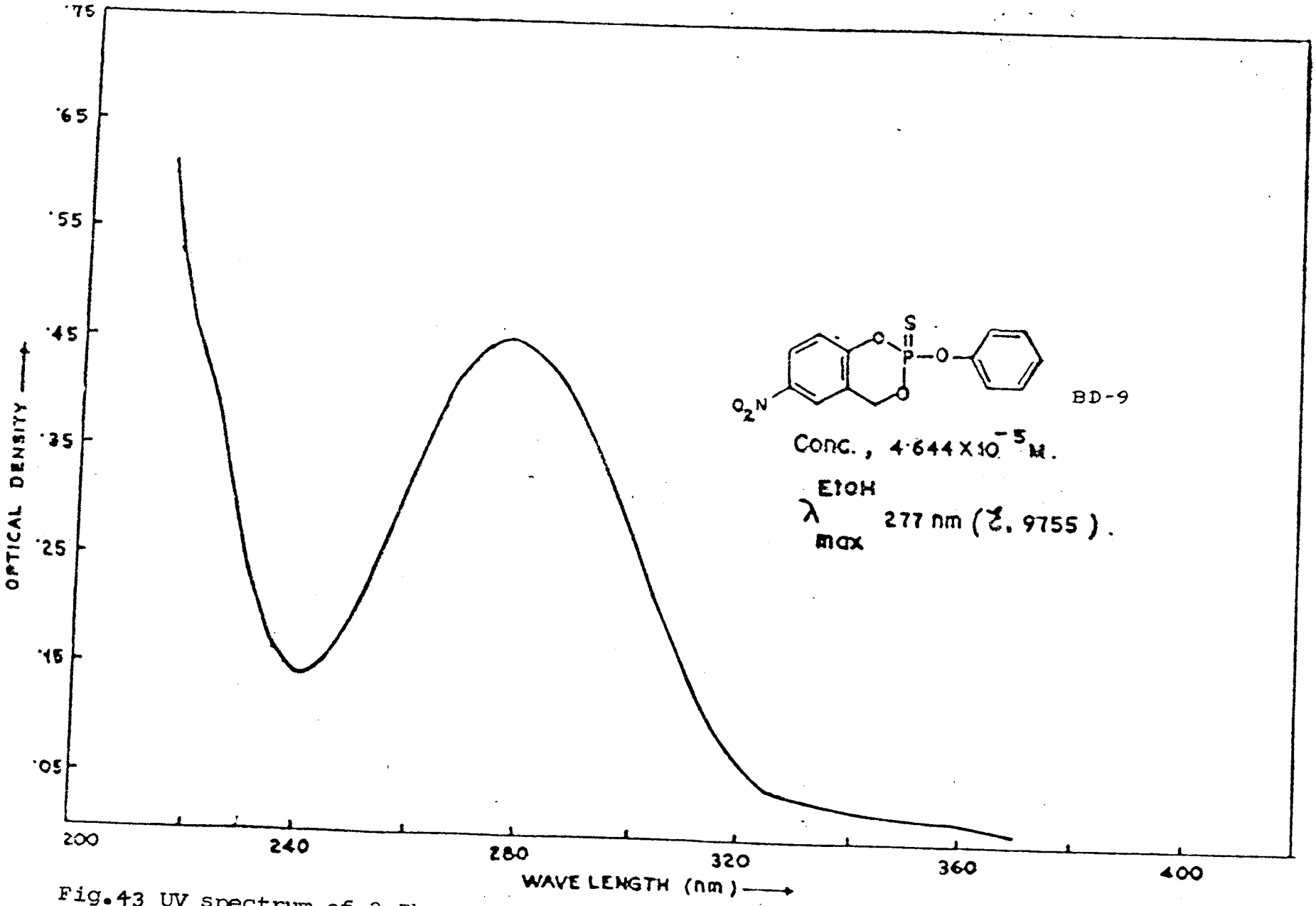


Fig.43 UV spectrum of 2-Phenoxy-6-nitro-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BD-9)

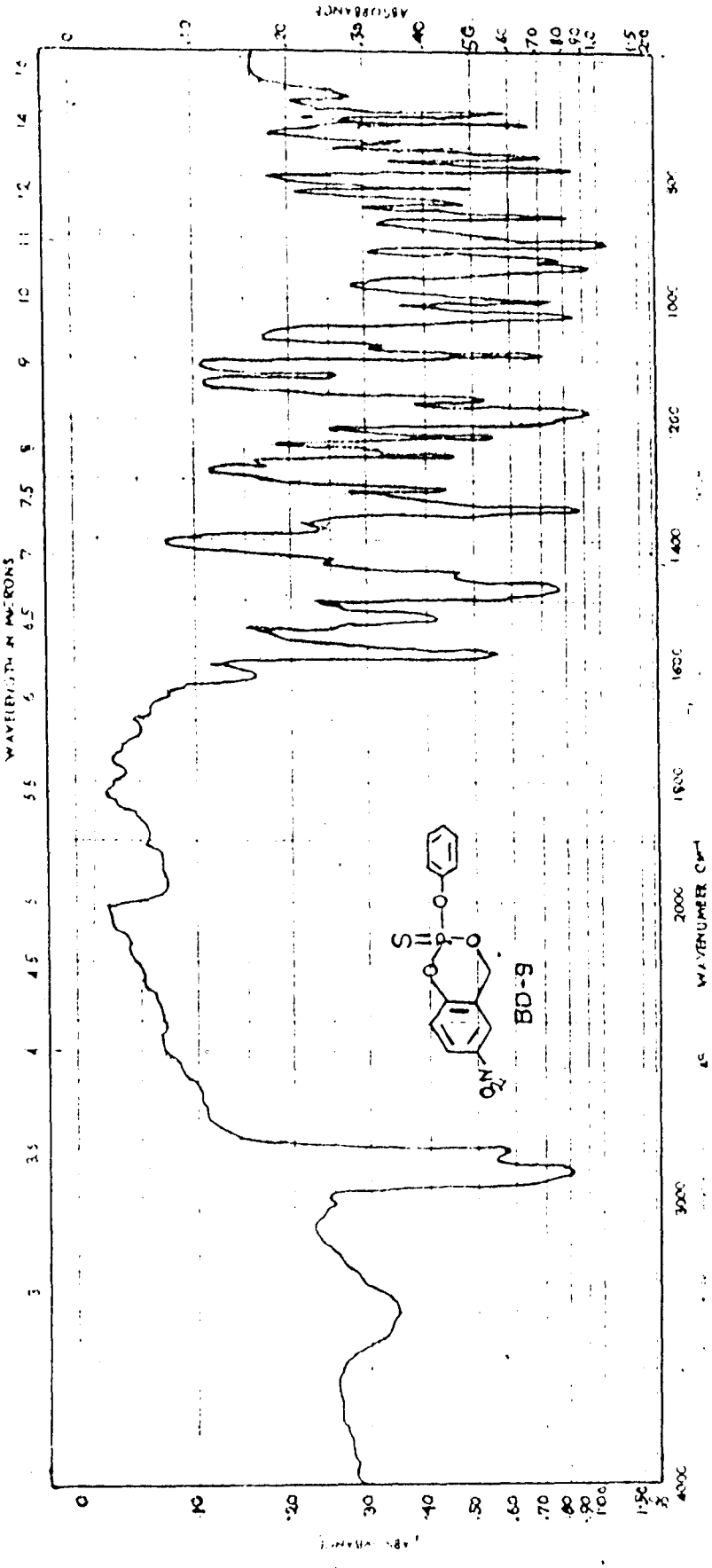


Fig.44 IR spectrum of 2-Phenoxy-6-nitro-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BD-9)

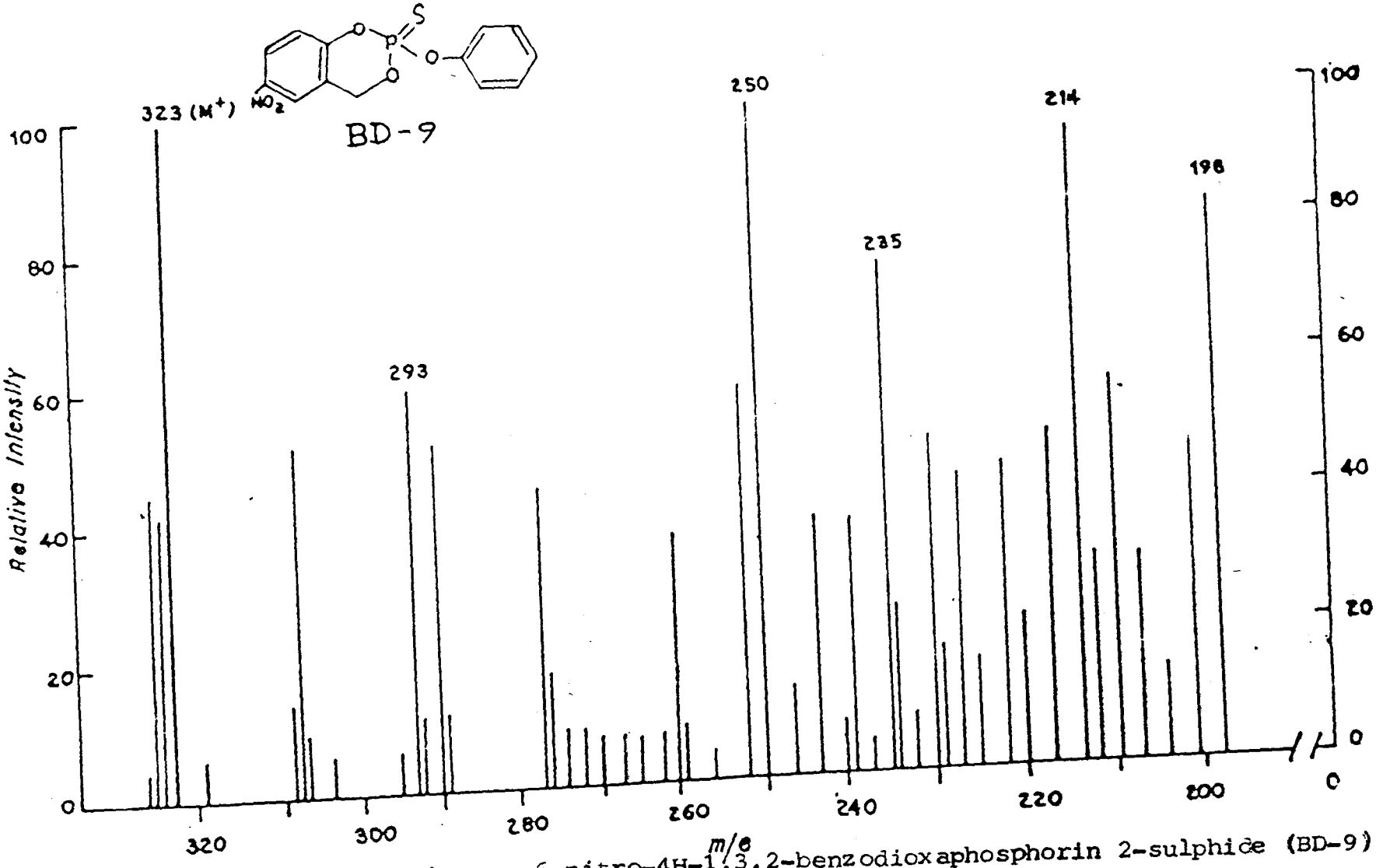


Fig.45 Mass spectrum of 2-Phenoxy-6-nitro-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BD-9)

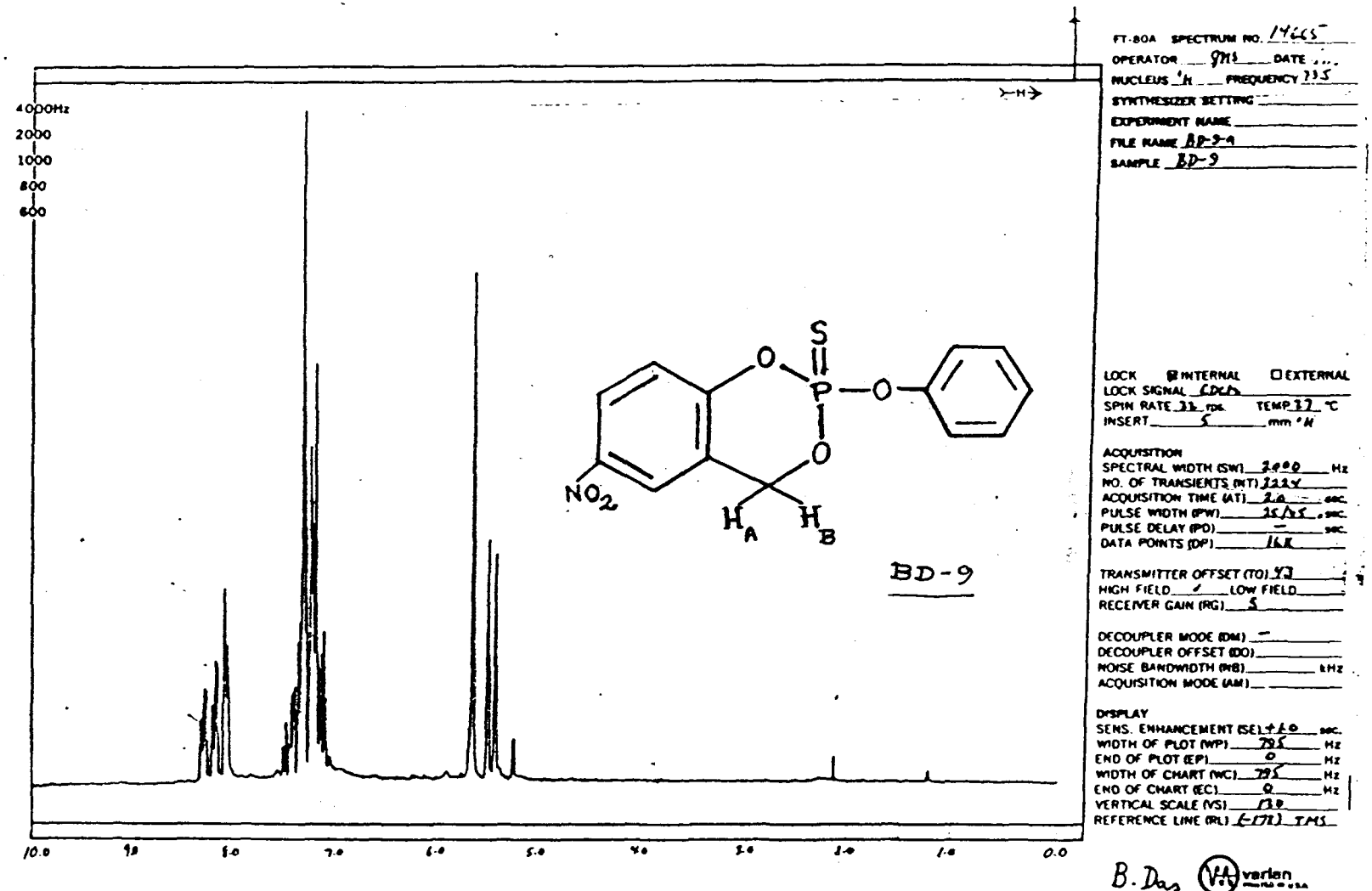


Fig.46 ^{1}H NMR spectrum of 2-Phenoxy-6-nitro-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BD-9)

(xi) 2-Diisobutylamido-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-sulphide (BD-25):

UV (Fig. 47)

λ_{max}^{EtOH} = 295 nm (ϵ = 17215)

IR (Fig.48)

1030 cm^{-1} (s)	P-O-C (alkyl);
1240 cm^{-1} (s) and 875 cm^{-1} (s)	P-O-C (aryl);
1515 cm^{-1} (vs)	asym. str. of nitro group;
1340 cm^{-1} (vs)	sym. str. of nitro group;
815 cm^{-1} (s)	P = S (I)
645 cm^{-1} (s)	P = S (II)
730 cm^{-1} (s)	P-N stretching

Mass (Fig. 49)

m/z	% RI
358 (M^+)	16.0
326	12.0
325	22.0
295 (base peak)	100.0
259	78.3
230	16.0
198	10.5
152	10.1

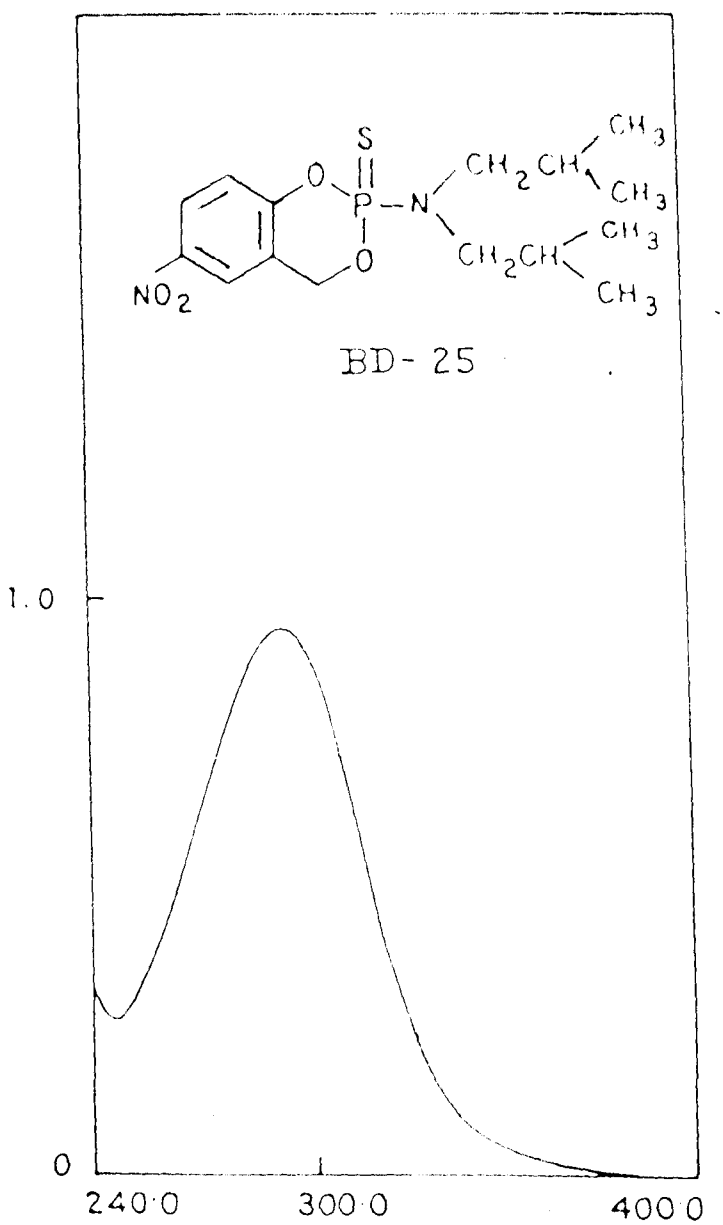


Fig.47 UV spectrum of 2-Diisobutylamido-6-nitro-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BD-25)

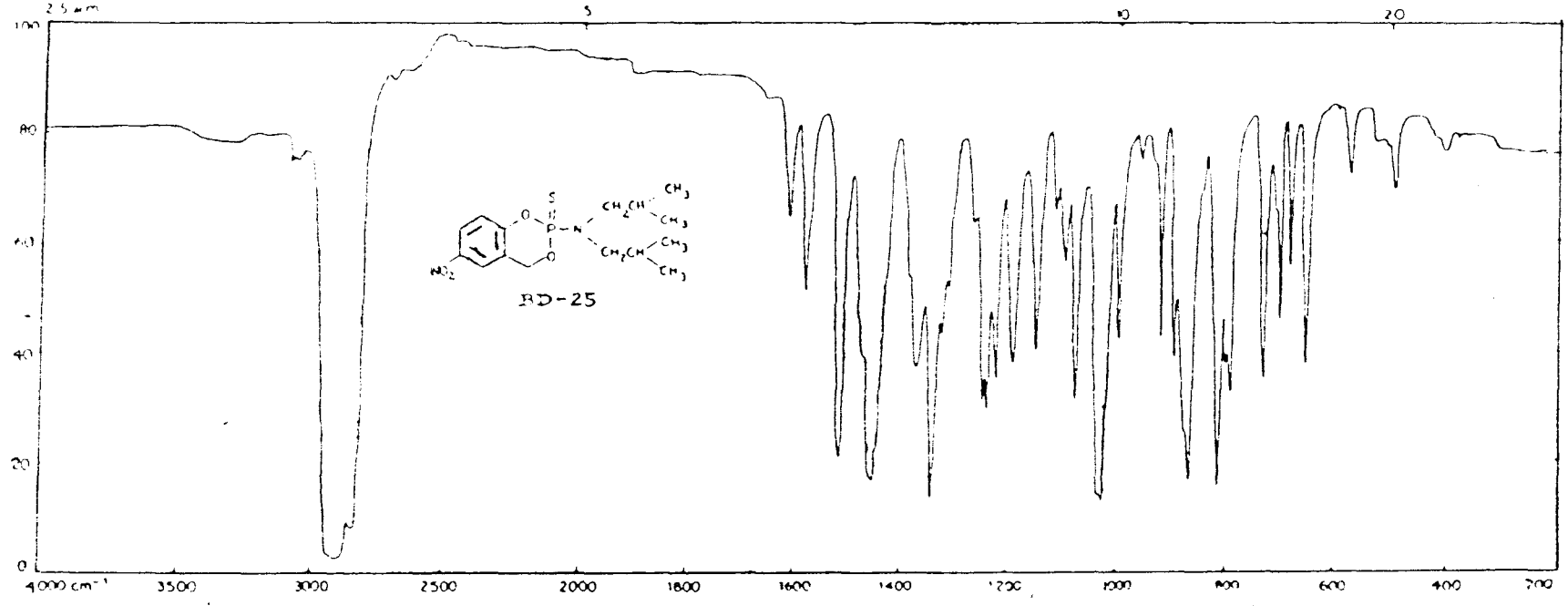


Fig 48 IR Spectrum of 2- Diisobutylamido- 6- Nitro- 4H-1,3,2 - benzodioxaphosphorin - 2 - Sulphide (BD - 25)

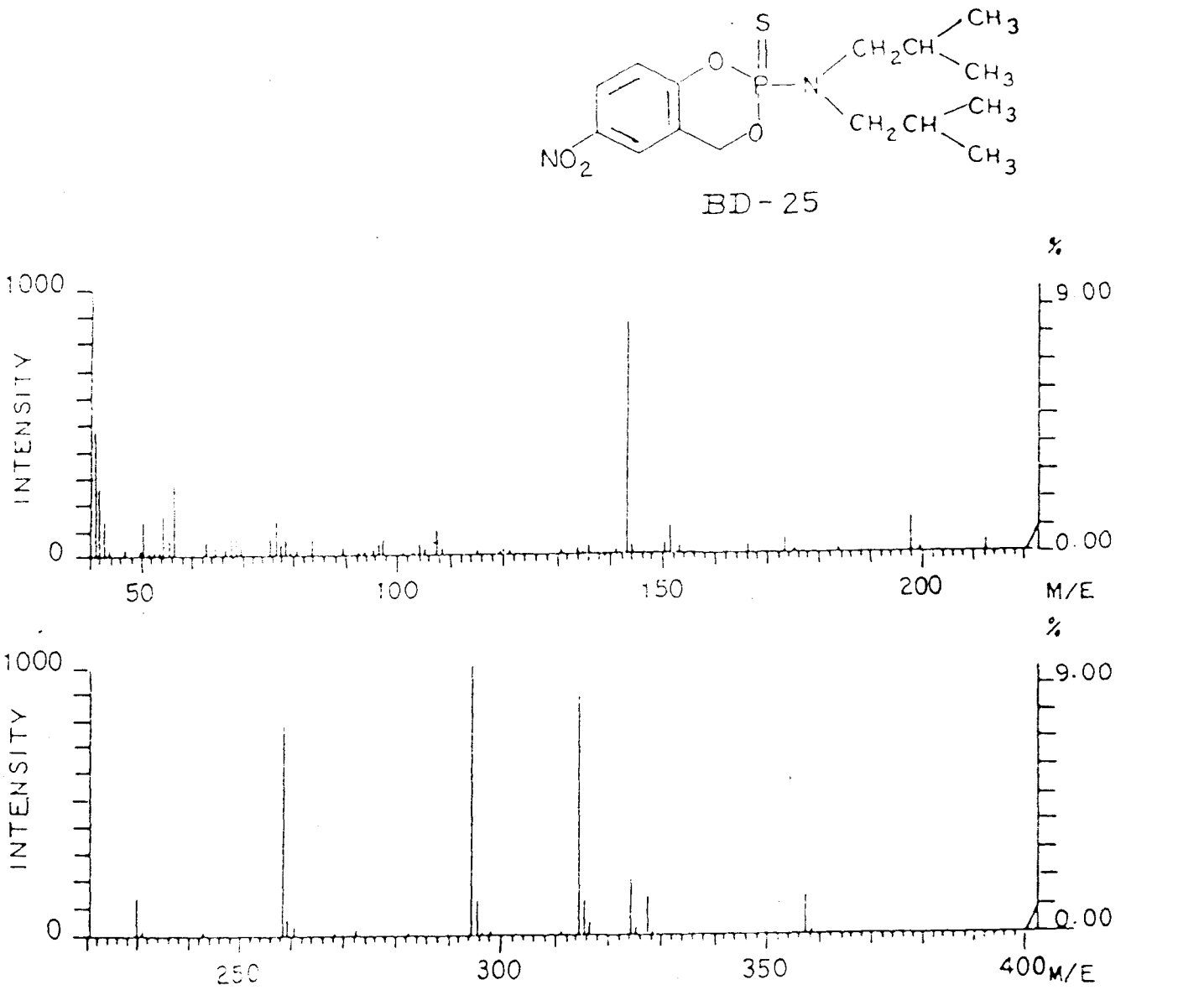


Fig.49 Mass spectrum of 2-Diisobutylamido-6-nitro-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BD-25)

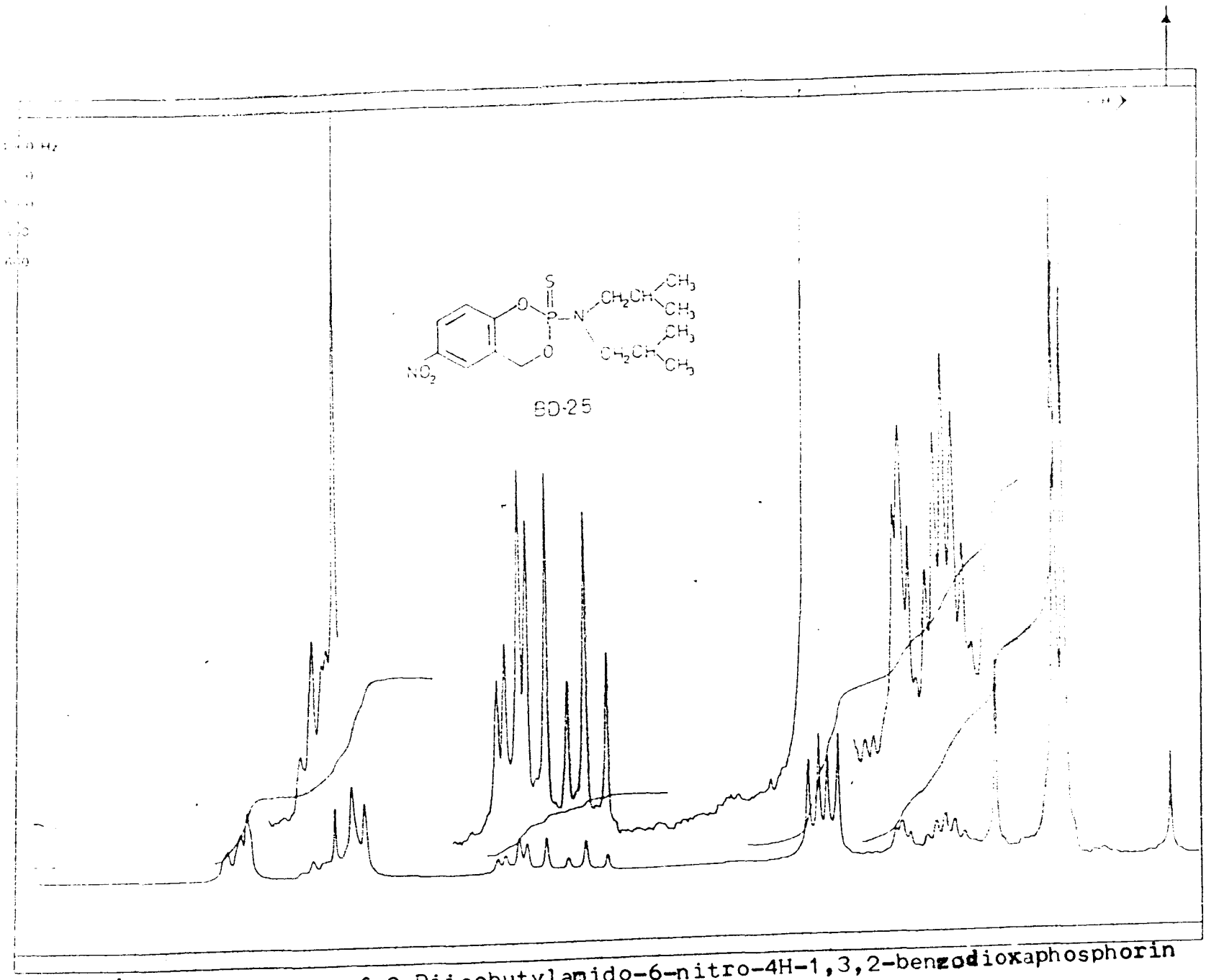


Fig.50 ^{1}H NMR spectrum of 2-Diisobutylamido-6-nitro-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BD-25)

^{1}H NMR $\delta(CDCl_3)$ ppm (Fig. 50)

0.95	(12H, doublet, due to four $-CH_3$ group);
1.70-2.45	(2H, multiplet due to two $-CH<$ group);
2.85-3.25	(4H, quartet, due to two $-CH_2$ group);
4.85-5.90	(2H, eight line multiplet due to $-CH_2$ group in benzodioxaphosphorin ring);
7.1, 8.05 and 8.1	(3H, due to aromatic hydrogen).

(xii) 2-Dibutylamido-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-sulphide (BD-29):

UV (Fig. 51)

λ_{max}^{EtOH} = 292 nm (ϵ = 9438)

IR (Fig. 51)

1030 cm^{-1} (s)	P-O-C (alkyl);
1245 cm^{-1} and 875 cm^{-1} (s)	P-O-C (aryl);
1520 cm^{-1} (vs)	asym. stretching of the nitro group;
1340 cm^{-1} (vs)	sym. stretching of the nitro group
815 cm^{-1} (s)	P = S (I)
650 cm^{-1} (s)	P = S (II)
730 cm^{-1} (s)	P-N stretching.

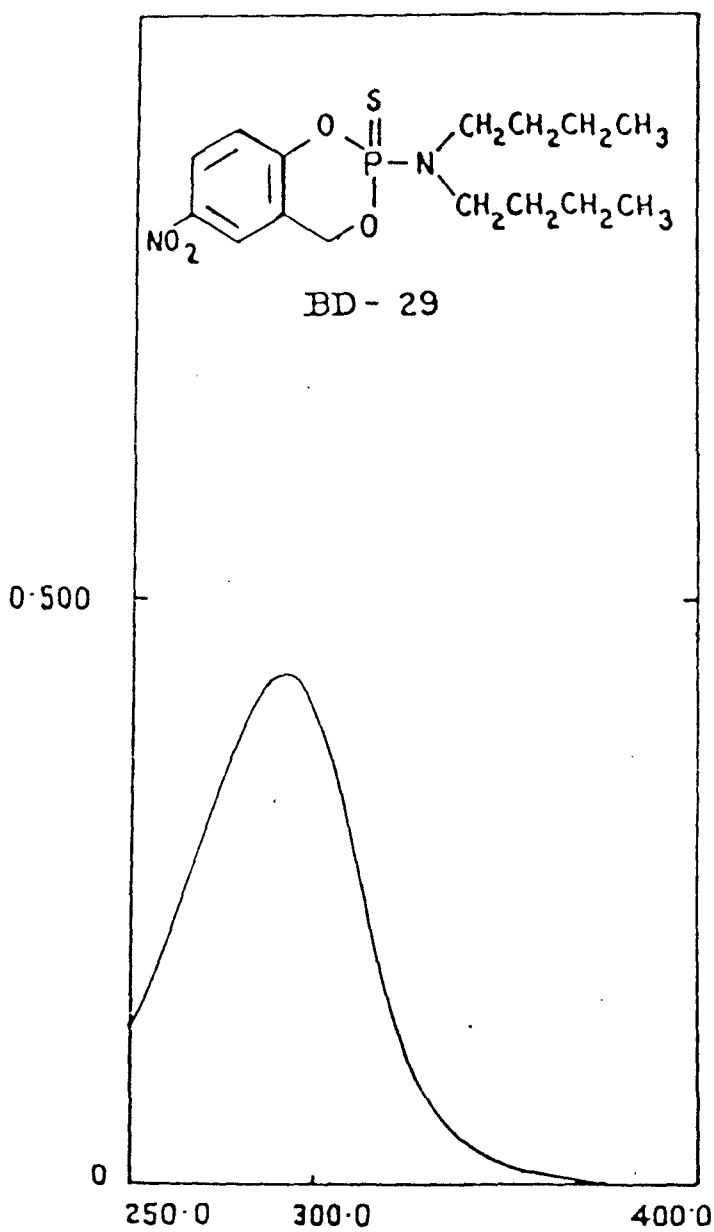


Fig.51 UV spectrum of 2-N,N-Dibutylamido-6-nitro-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BD-29)

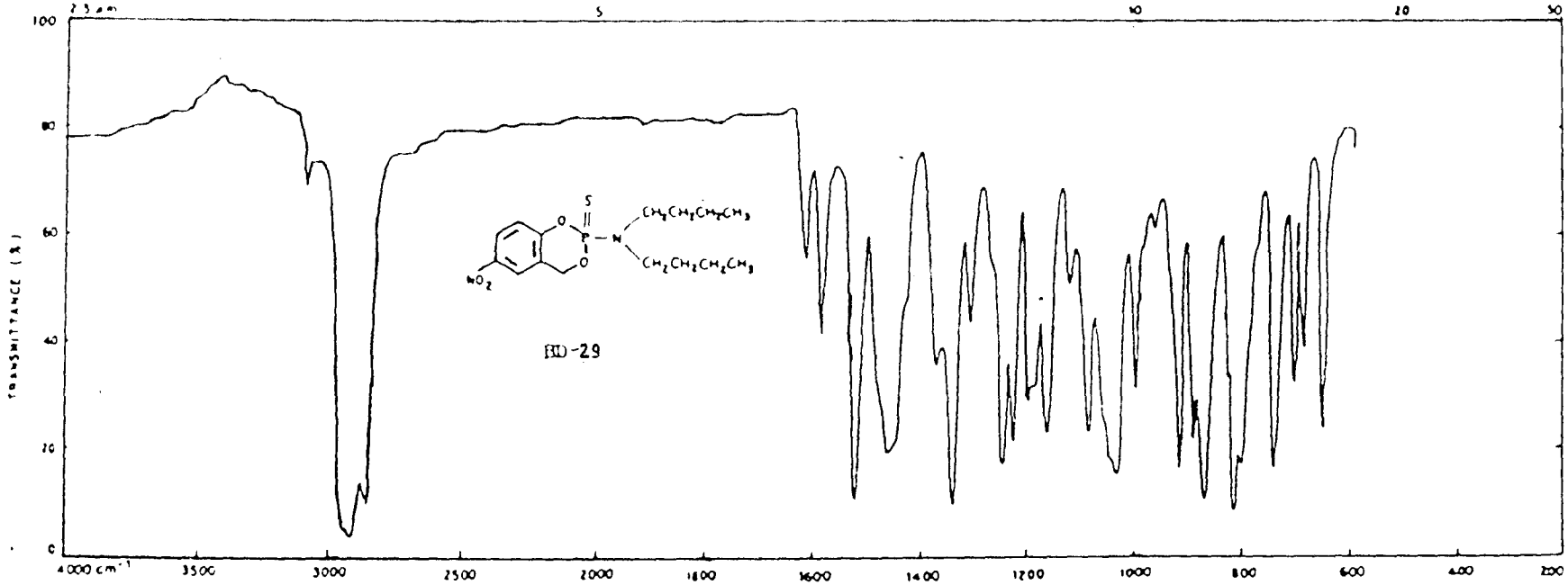


Fig.52 IR spectrum of 2-N,N-Dibutylamido-6-nitro-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BD-29)

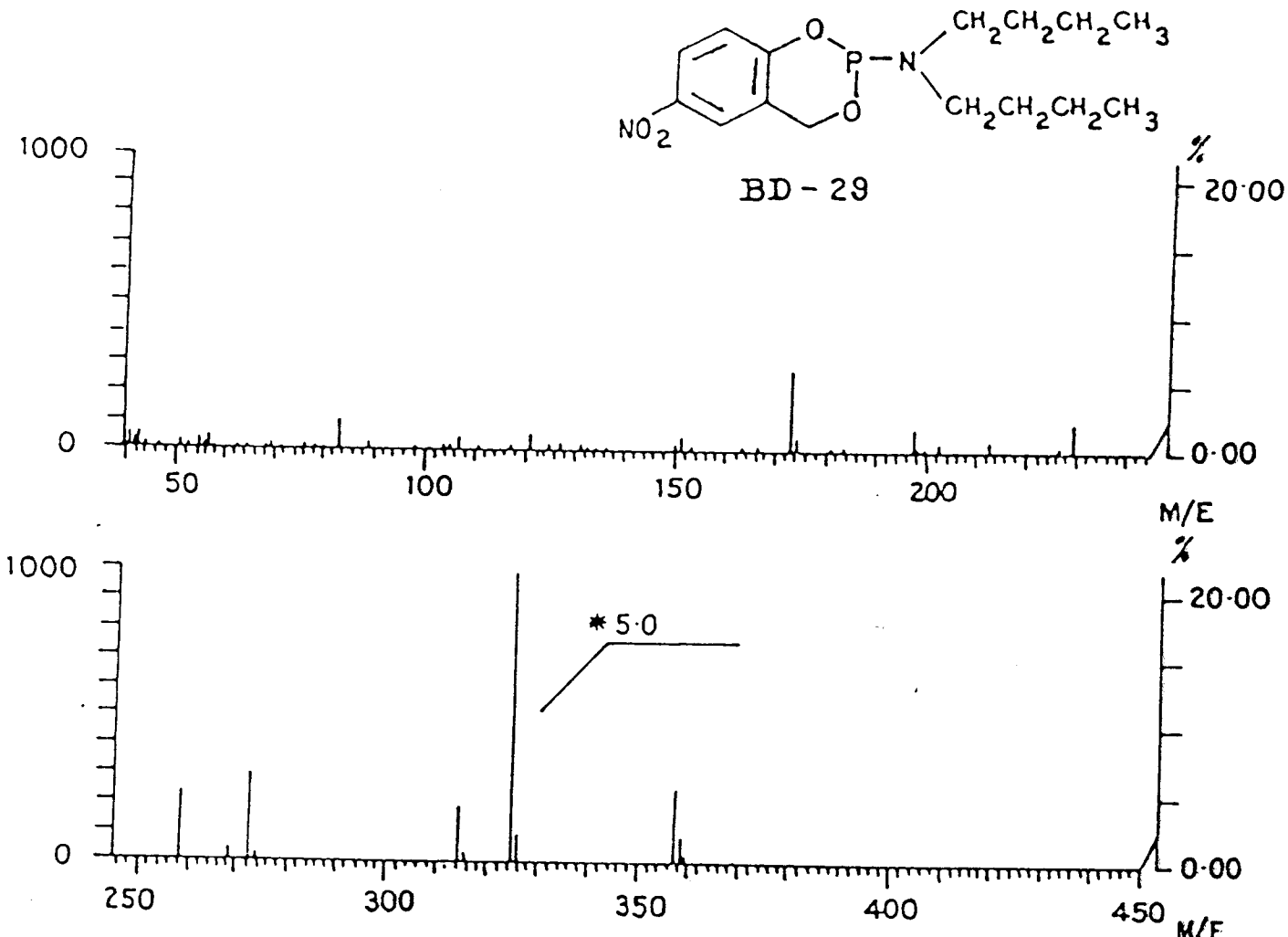


Fig.53 Mass spectrum of 2-N,N-Dibutylamido-6-nitro-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BD-29)

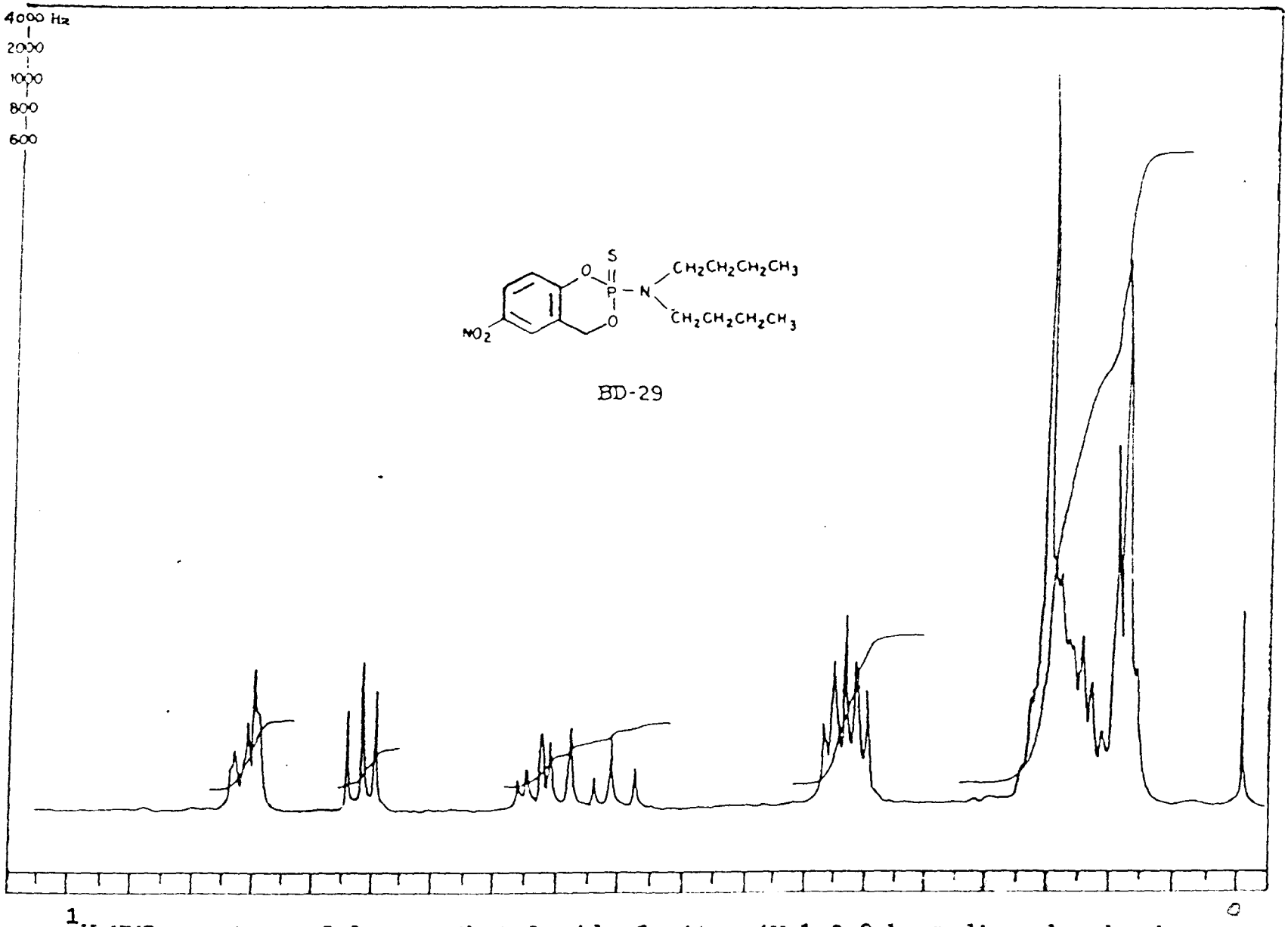


Fig. 54 ^{1}H NMR spectrum of 2-N,N-Dibutylamido-6-nitro-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BD-29)

Mass (Fig. 53)

m/z	% RI
350 (M^+)	5.4
325 (Base peak)	100.0
315	19.9
273	31.0
259	29.6
198	10.0

^{1}H NMR δ ($CDCl_3$) ppm (Fig. 54)

0.95	(6H, doublet due to two $-CH_3$ group);
1.15-1.75	(8H, multiplet, due to two $-CH_2CH_2-$ group);
2.45-3.40	(4H, multiplet, due to two $-CH_2-$ group);
4.85-5.81	(2H, eight line multiplet due to $-CH_2$ gr. in the benzodioxaphosphorin ring);
7.07, 8.0 and 8.15	(3H, due to three aromatic hydrogens).

2(b) Discussion:

IR spectra

The IR spectra of the (nitro/bromo/chloro) saligenin cyclic phosphoramidothionates have been analysed according to Thomas[20], Bellamy[21], Colthup[22] et al and Das[23]. The important IR bands are summarised below:

1000-1070 cm^{-1} (s)	P-O-C (alkyl);
1185-1260 cm^{-1} (s) and	
875-950 cm^{-1} (s)	P-O-C (aryl);
1515-1530 cm^{-1} (s)	asym. str. of nitro group;
1340-1345 cm^{-1} (s)	sym. str. of nitro group;
785-830 cm^{-1}	P = S (I);
645-665 cm^{-1}	P = s (II).

The thiono group is characterised by two IR absorption bands with frequencies in the normal ranges given by Thomas[20], as both are not observed in phosphoramidate; among these two frequencies, the lower frequency band P = S (II) is assigned to 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. In the nitro-saligenin cyclic alkoxy, ~~phenoxy~~ compounds, Das et al[24] have observed the two bands in the region : 650-675 cm^{-1}, P = S (II); and 780-820 cm^{-1}, P = S (I). It may be concluded that in these compounds the frequency of band I is only slightly affected by substitution (alkylamido, alkoxy ~~or phenoxy~~ group to the phosphorus atom) and that of band II is affected to a greater extent. From the above data it can be observed that neither of the two bands show any

systematic shifts which reflect changes in the inductive properties of the substituents, and this is not unexpected if they do indeed arise from mixed modes. It has also been observed that the P = S(I) band is of medium intensity while the intensity of the P = S (II) band is variable. This has also been reported by Thomas[20].

The P-O-C (alkyl) group is characterised by a strong absorption band whose frequency is in the region of 1000-1070 cm^{-1} while the band due to P-O-C (aryl) group is found near 1200 cm^{-1} for all compounds (doublet in the range 1150-1240 cm^{-1}). This band is always accompanied by a second absorption band which has been attributed to either the symmetric stretch of P-O-C (aromatic) system, the antisymmetric mode being that at 1200 cm^{-1}, or to a separate P-O stretch which is not so coupled. Thomas[20] strongly favours the latter explanation which is supported by the persistance of this band in both P-O-P and P-OH compounds, and by the fact that in the latter the frequency is a linear function of the π-values (where, π is 'phosphorus-induction constant[25] for substituent groups), of the substituents. This band lies between 870-910 cm^{-1} for all compounds; the frequency grange quoted is that of the strongest band in this region.

The bands present in the ranges of 1515-1530 cm^{-1} and 1340-1345 cm^{-1} are due to asymmetric and symmetric stretch of nitro group respectively[21]. The bands present at 1615-1620 cm^{-1} and 1580-1590 cm^{-1} are due to "quadrant stretching" C = C vibration of the aromatic[22] ring.

2(c) Mass spectra:

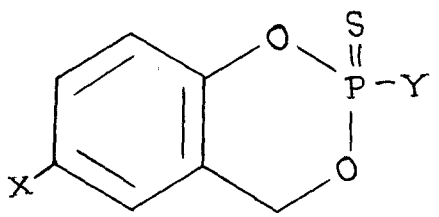
The mass spectra of these compounds have been analysed according to Cooks and Gerrard[26], Jörg et al[27], Damico et al[28,29], Gills and Occolowitz[30], Djerassi et al[31].

The mass spectra of twelve compounds have been taken. All compounds except BD-9 show parent molecular ion peaks (M^+). Fragmentation by loss of SH radical is important.

6-chloro/bromo saligenin cyclic phosphoramidothionates also show $(M + 2)^+$ ion peaks. For chloro compounds (CL-6, CL-10) the $(M + 2)^+$ ion peaks are approximately one-third in intensity of the parent molecular ion peak (M^+) because of the presence of a molecular ion containing the ^{37}Cl isotope. All the chlorine containing fragments show $(fragment + 2)^+$ ion peaks are nearly one-third in intensity of the fragment ion peaks. For bromo compounds (BR-6, BR-10, BR-27) the $(M + 2)^+$ ion peaks are almost equal in intensity to the parent molecular ion peaks because of the presence of a molecular ion containing ^{81}Br isotope. Again all the bromine containing fragments show $(Fragment + 2)^+$ ion peaks of almost equal intensity. These results prove the presence of one chlorine or one bromine atom in the 6-chloro/bromo saligenin cyclic phosphoramidothionates.

Table - 2

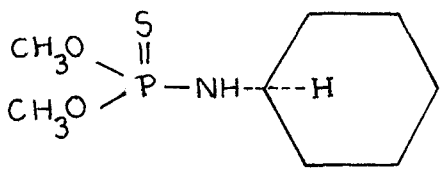
Base peaks of 2-Alkoxy/Alkyl amido 6-chloro/bromo nitro-4H-1,3,2-benzodioxaphosphorin-2-sulphides



Code No.	X	Y	Base peak	Ion
CL-6	Cl	N,N-Diisobutylamido	304	$C_{12}H_{16}O_2ClNPS^+$
CL-10	Cl	N,N-Dibutylamido	314	$C_{15}H_{22}ClO_2NP^+$
CL-24	Cl	N-Butylamido	258.5	$C_{11}H_{14}O_2NClP^+$
BR-6	Br	N,N-Diisobutylamido	348	$C_{12}H_{16}BrO_2NPS^+$
BR-10	Br	N,N-Dibutylamido	358	$C_{15}H_{22}O_2PNBr^+$
BR-24	Br	N-Butylamido	302	$C_{11}H_{14}O_2NBrP^+$
BR-27	Br	N-Hexylamido	184	$C_7H_5OBr^+$
BD-1	NO_2	Methoxy-ethoxy	58	$C_3H_6O^+$
BD-5	NO_2	Isopropoxy	246	$C_7H_5NPSO_5^+$
BD-9	NO_2	Phenoxy	323	$C_{13}H_{10}NO_5PS^+$
BD-25	NO_2	N,N-Diisobutylamido	295	$C_{13}H_{16}N_2O_4P^+$
BD-29	NO_2	N,N-Dibutylamido	325	$C_{15}H_{22}N_2O_4P^+$

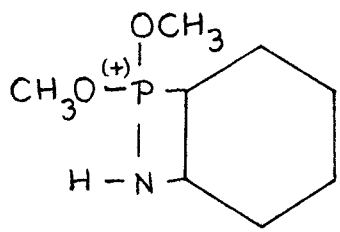
The base peaks of different compounds are presented in table - 2.

Cooks and Gerrard[26] reported that compounds of the type (I) formed the base peak by the loss of SH radical directly from the molecular ion. By deuteration of the methyl group and amino group it was shown that the hydrogen of SH is obstracted from the cyclohexyl ring but not from the N-H entity. There was



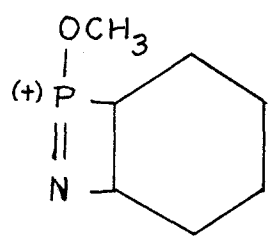
(I)

no preliminary hydrogen shift to sulphur. They postulated structure (II) for the product ion.



(II)

By specific loss of amino hydrogen, (II) further loss of CH_3OH giving an ion for which they postulated the structure (III).



(III)

These two structures (II and III) are supported by analogous fragmentation of the related compounds.

Following Cooks and Gerrard[26], we postulated the mass fragmentation process for the different compounds.

CL-6 (Scheme - I)

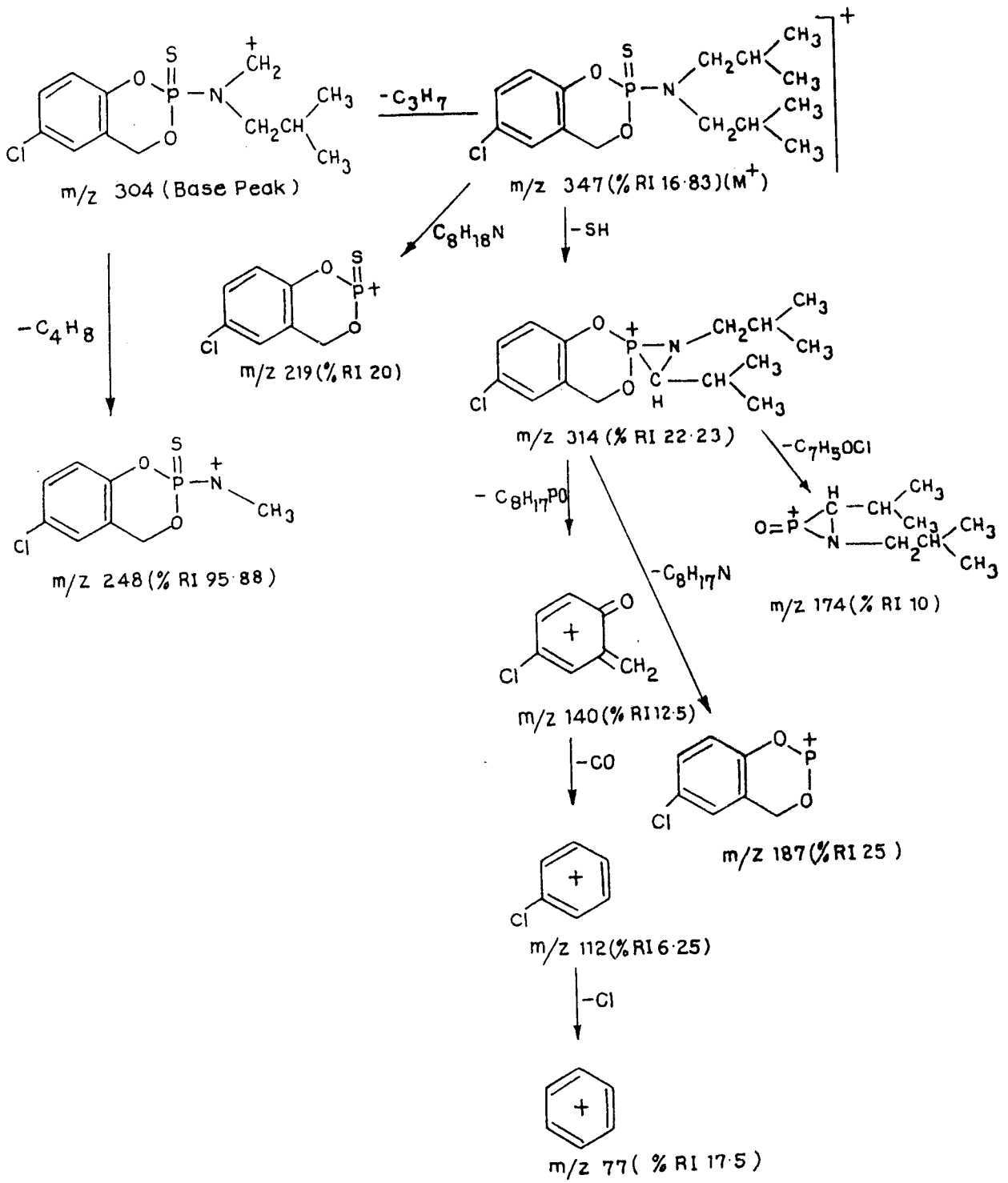
The mass spectra of compound CL-6, exhibits the fragmentation m/z 304 as the base peak by the loss of C_3H_7 from the molecular ion m/z 347. The ion (m/z 314, % RI 22.23) is formed by the direct elimination of SH from the molecular ion. The ion (m/z 248, % RI 95.88) resulting from the loss of C_4H_8 molecule from the base peak is obtained. The other major ions are (m/z 219, % RI 20.0), (m/z 187, % RI 25.0), (m/z 174, % RI 10.0), (m/z 140, % RI 12.5), (m/z 112, % RI 6.25) and (m/z 77, % RI 17.5).

CL-10 (Scheme-2)

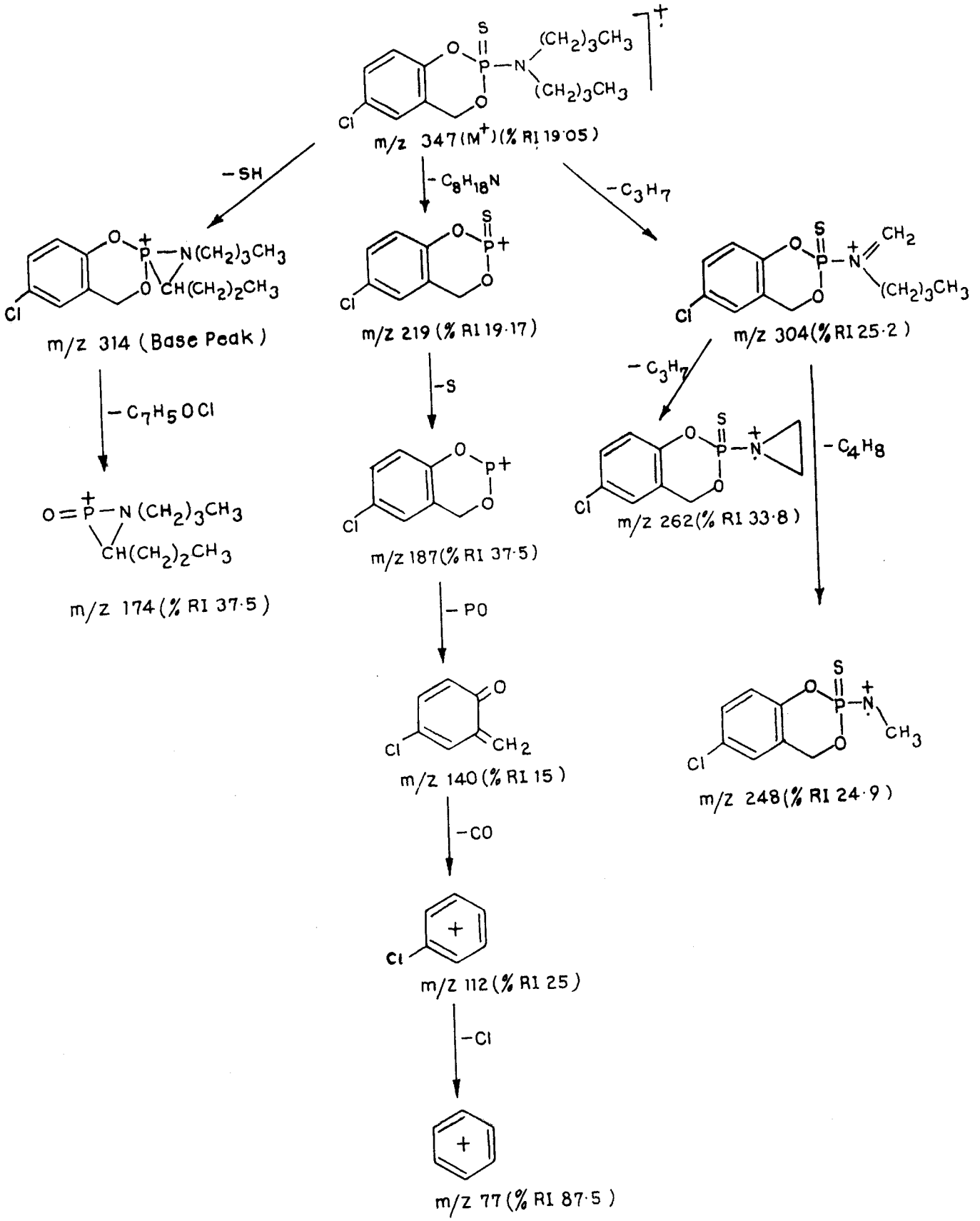
For compound CL-10, molecular ion peak is (m/z 347, % RI 19.05). The commonest fragmentation involves the loss of SH from the molecular ion which gives rise to the base peak m/z 314. The other major peaks are at (m/z 304, % RI 25.20), (m/z 262, % RI 33.80), (m/z 248, % RI 24.90), (m/z 219, % RI 19.17), (m/z 187, % RI 37.50), (m/z 174, % RI 37·50), (m/z 112, % RI 25.0) and (m/z 77, % RI 87.50).

CL-24 (Scheme-3)

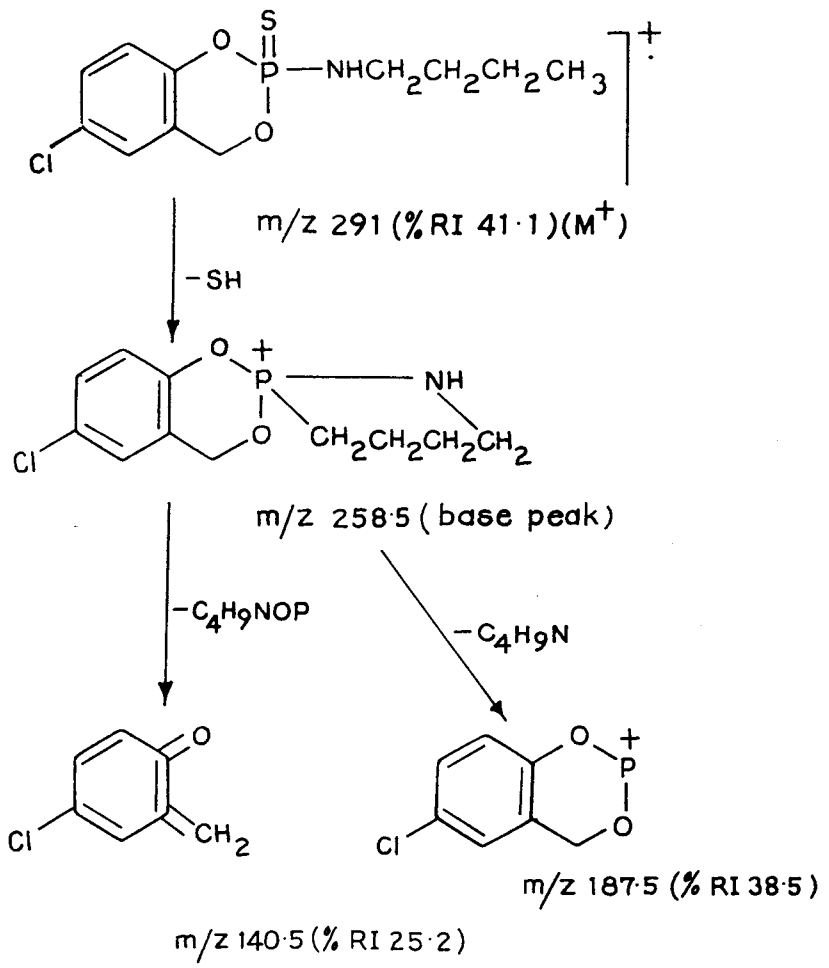
This compound shows m/z 258.5 ion as the base peak by the direct elimination of SH from the M^+ ion peak (m/z 291.5, % RI 41.1). The ion (m/z 140.5, % RI 25.2) and the ion (m/z 187.5, % RI 38.5) are formed by the direct loss of C_4H_9NOP and C_4H_9N respectively from the base peak ion.



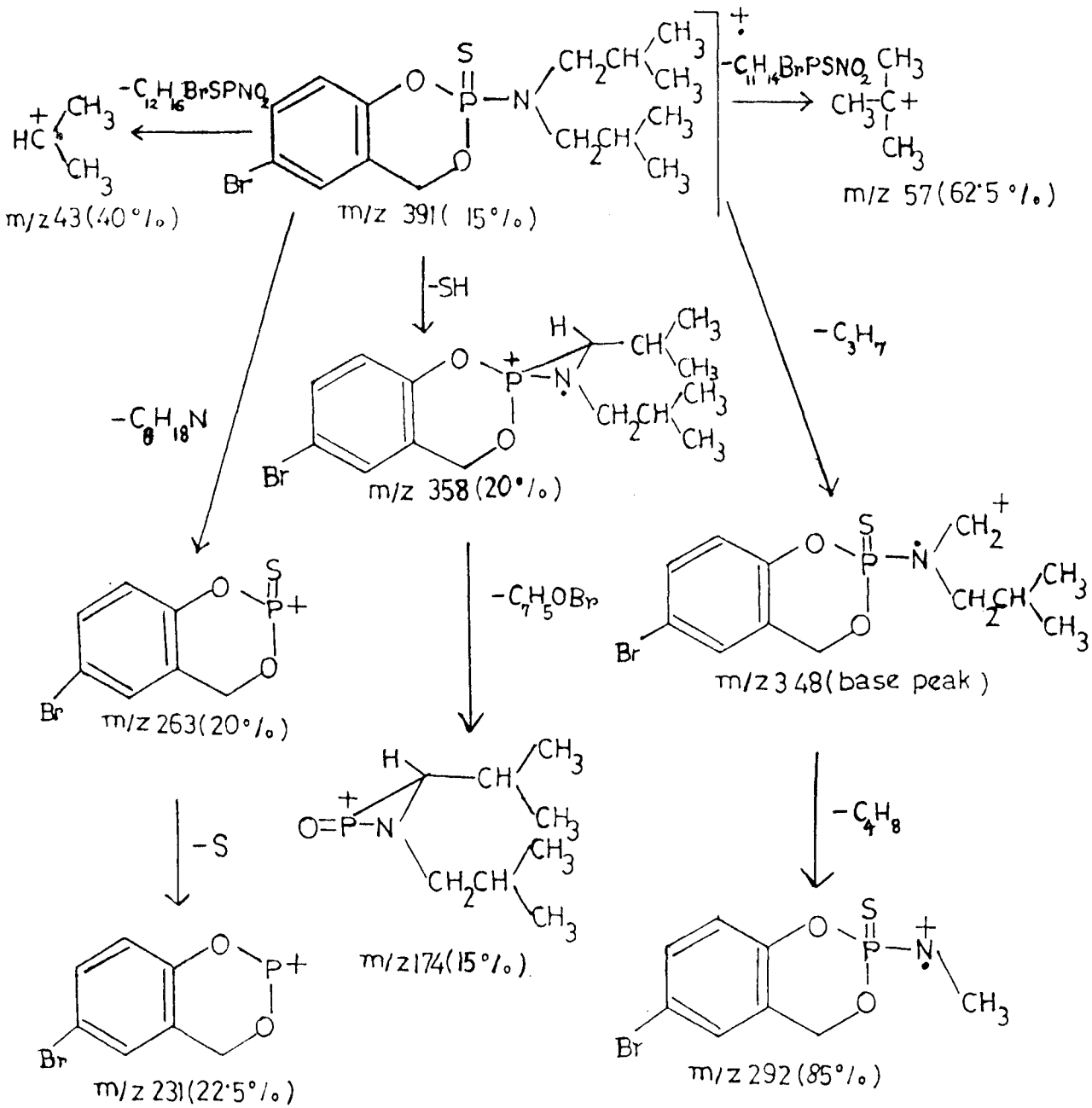
Mass fragmentation of CL- 6 (Scheme - 1)



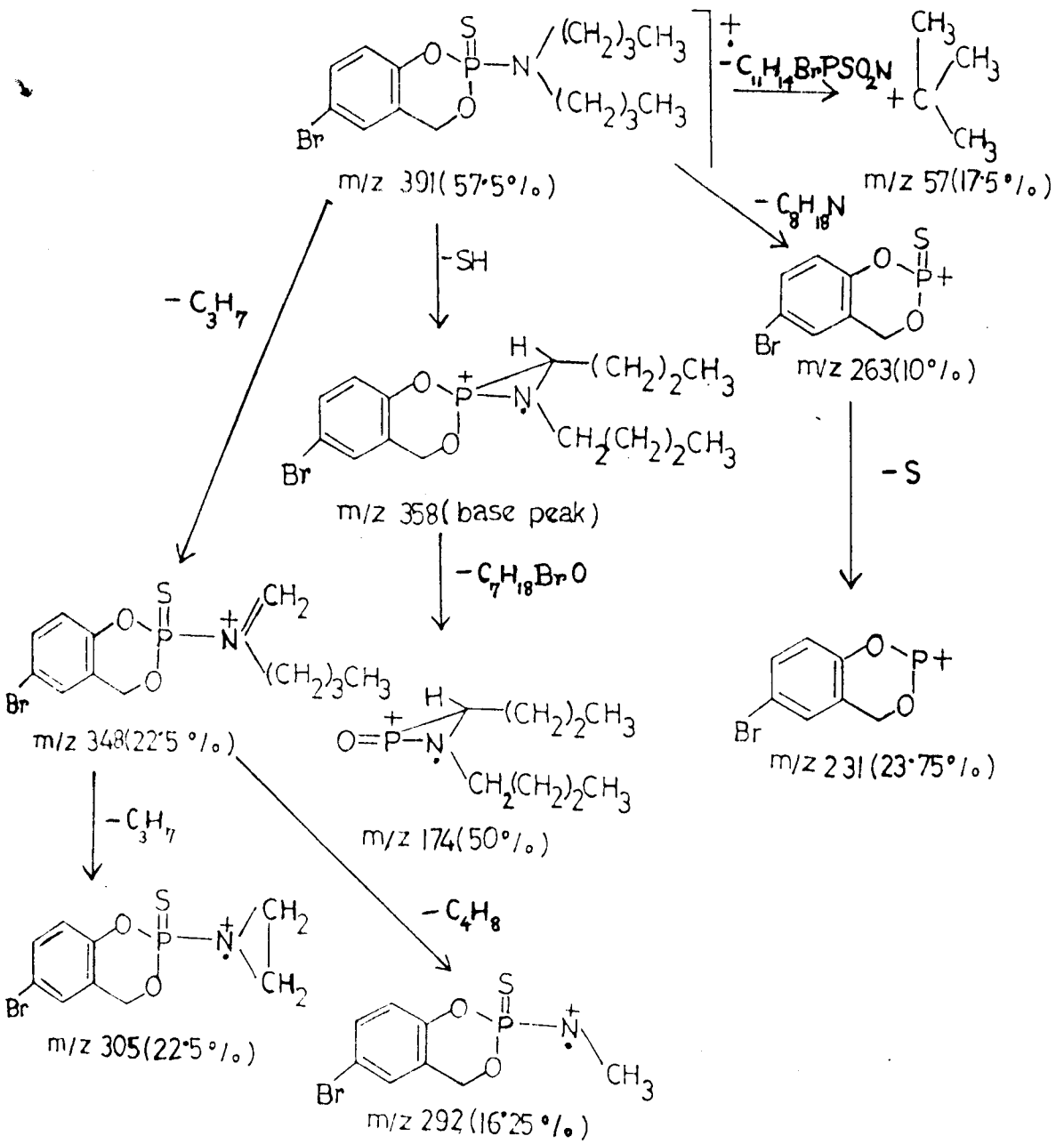
MASS FRAGMENTATION OF CL-10 (SCHEME - 2)



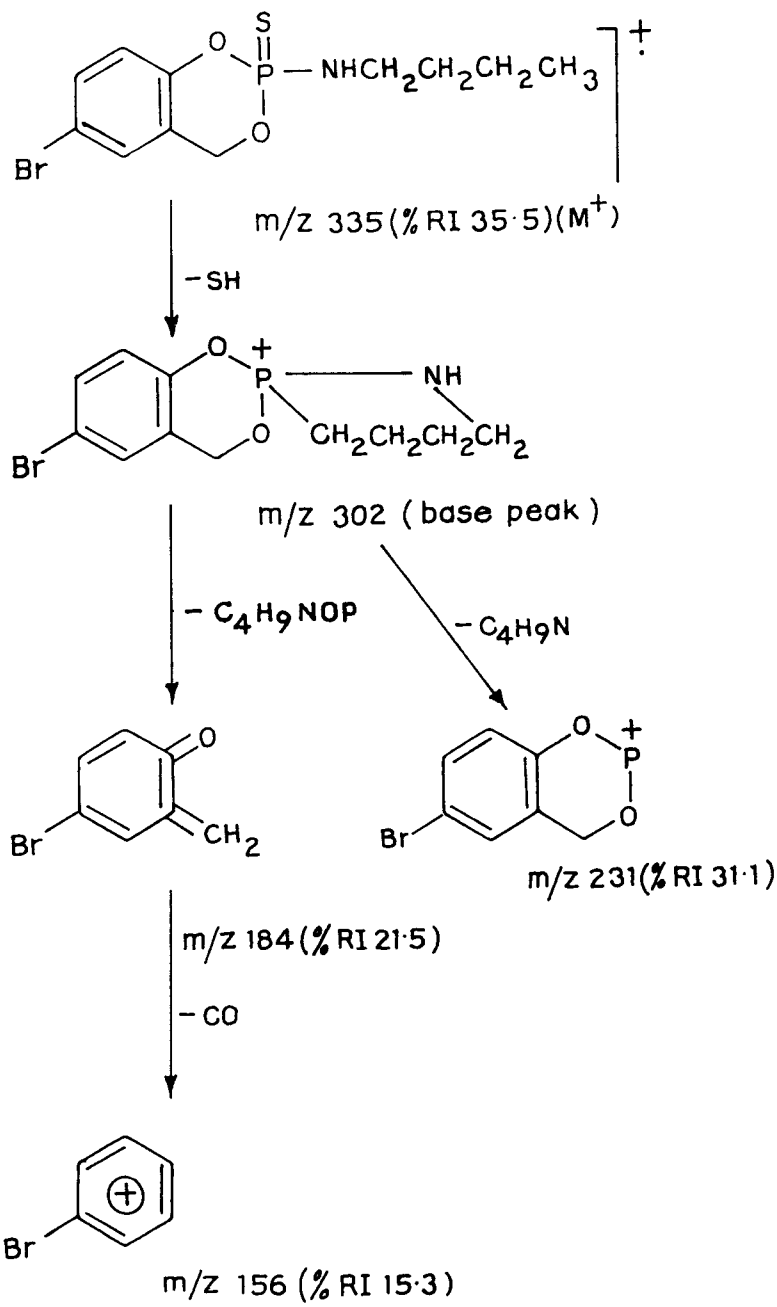
MASS FRAGMENTATION OF CL-24 (SCHEME-3)



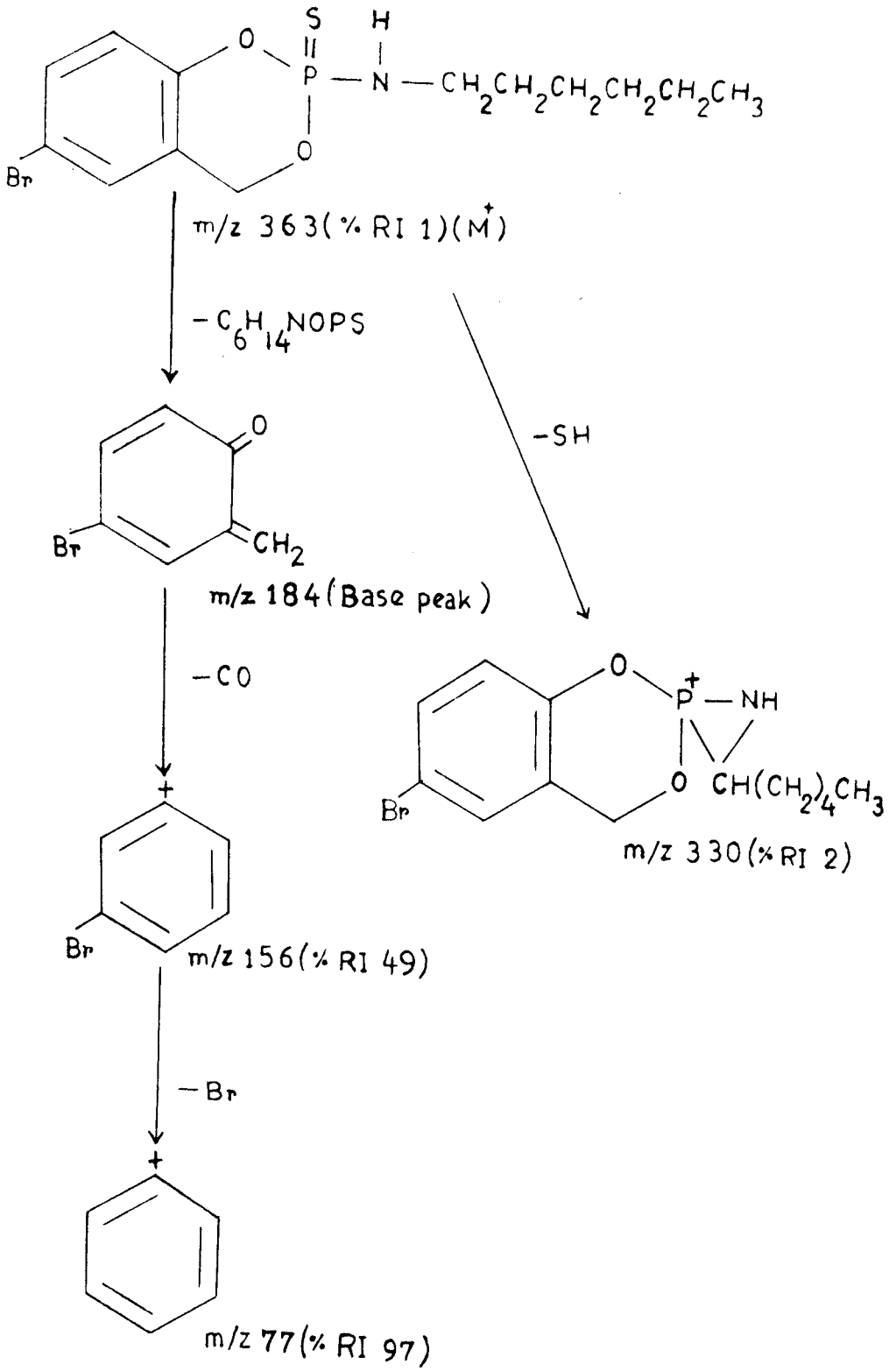
Mass fragmentation of BR-6 (Scheme-4)



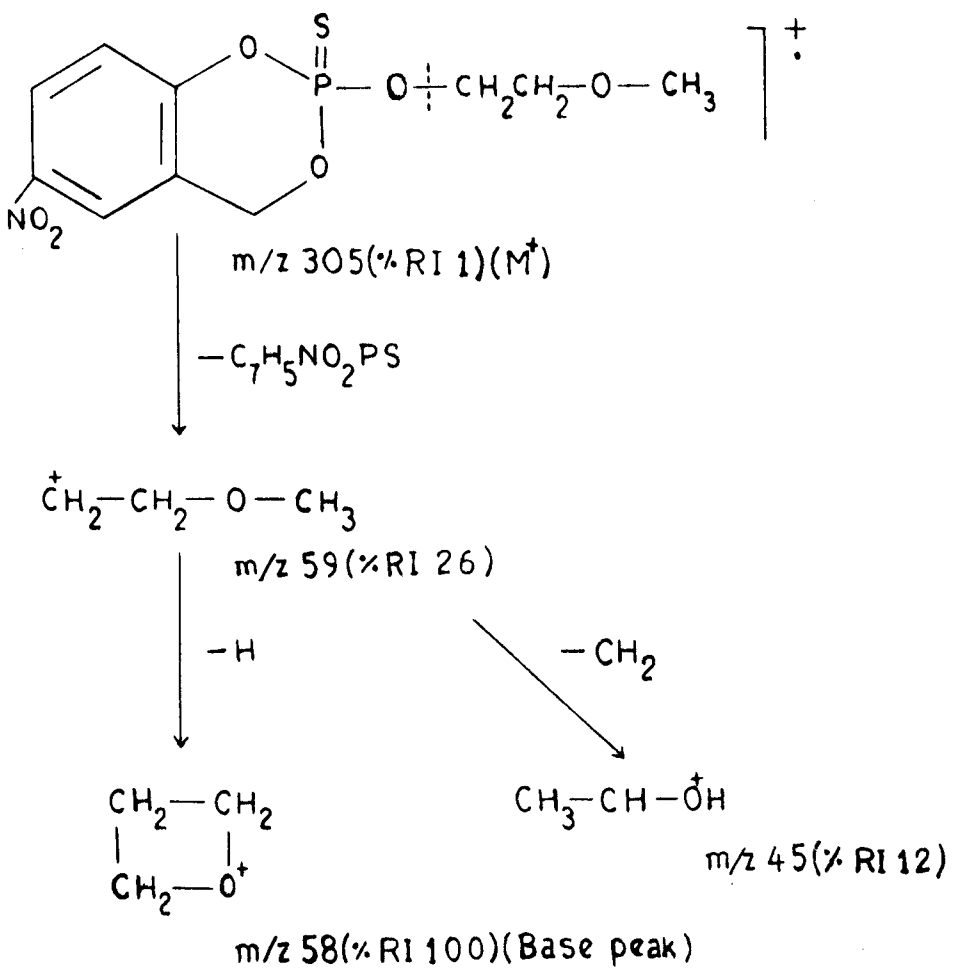
Mass fragmentation of BR-10 (Scheme - 5)



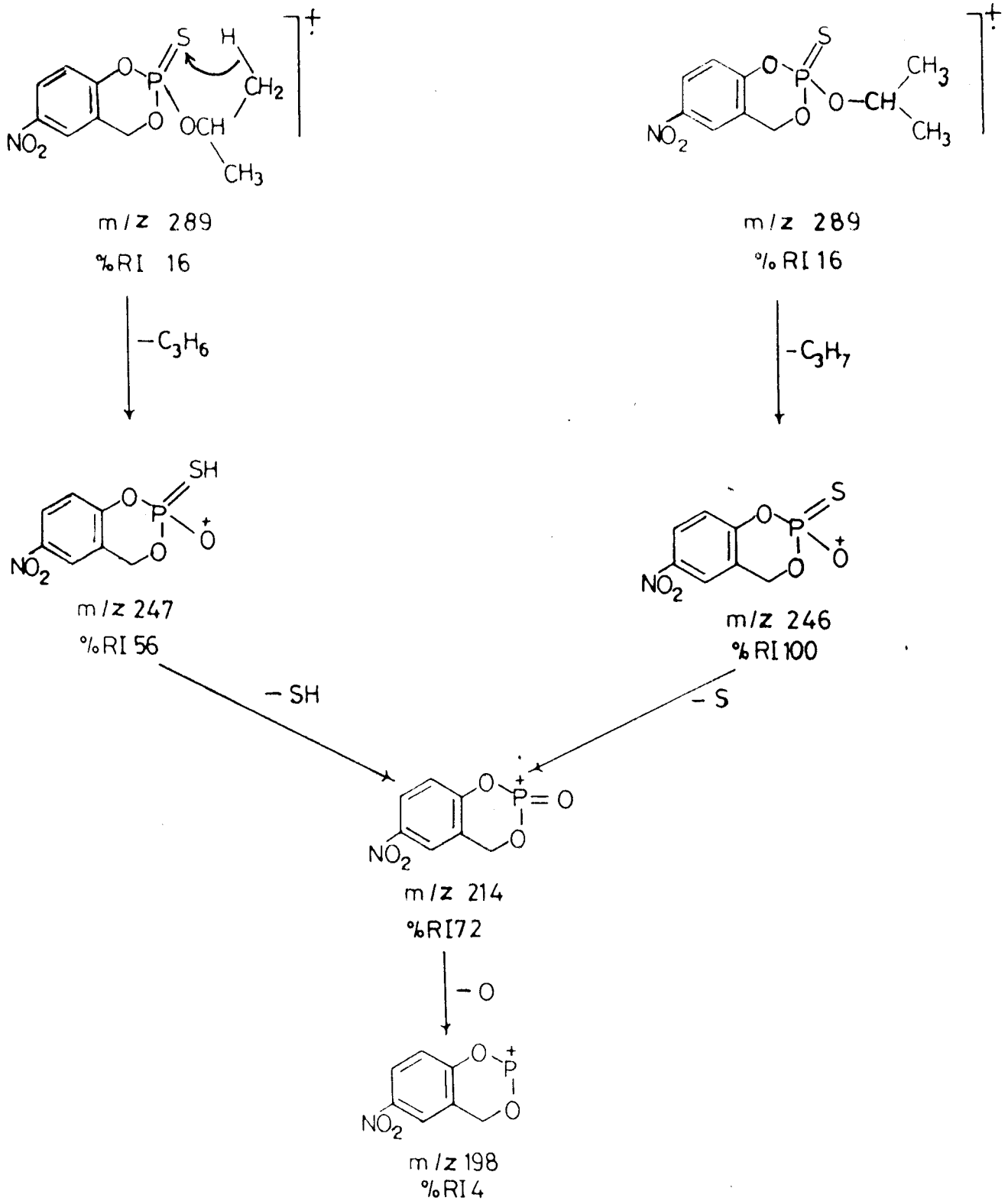
MASS FRAGMENTATION OF BR- 24 (SCHEME- 6)



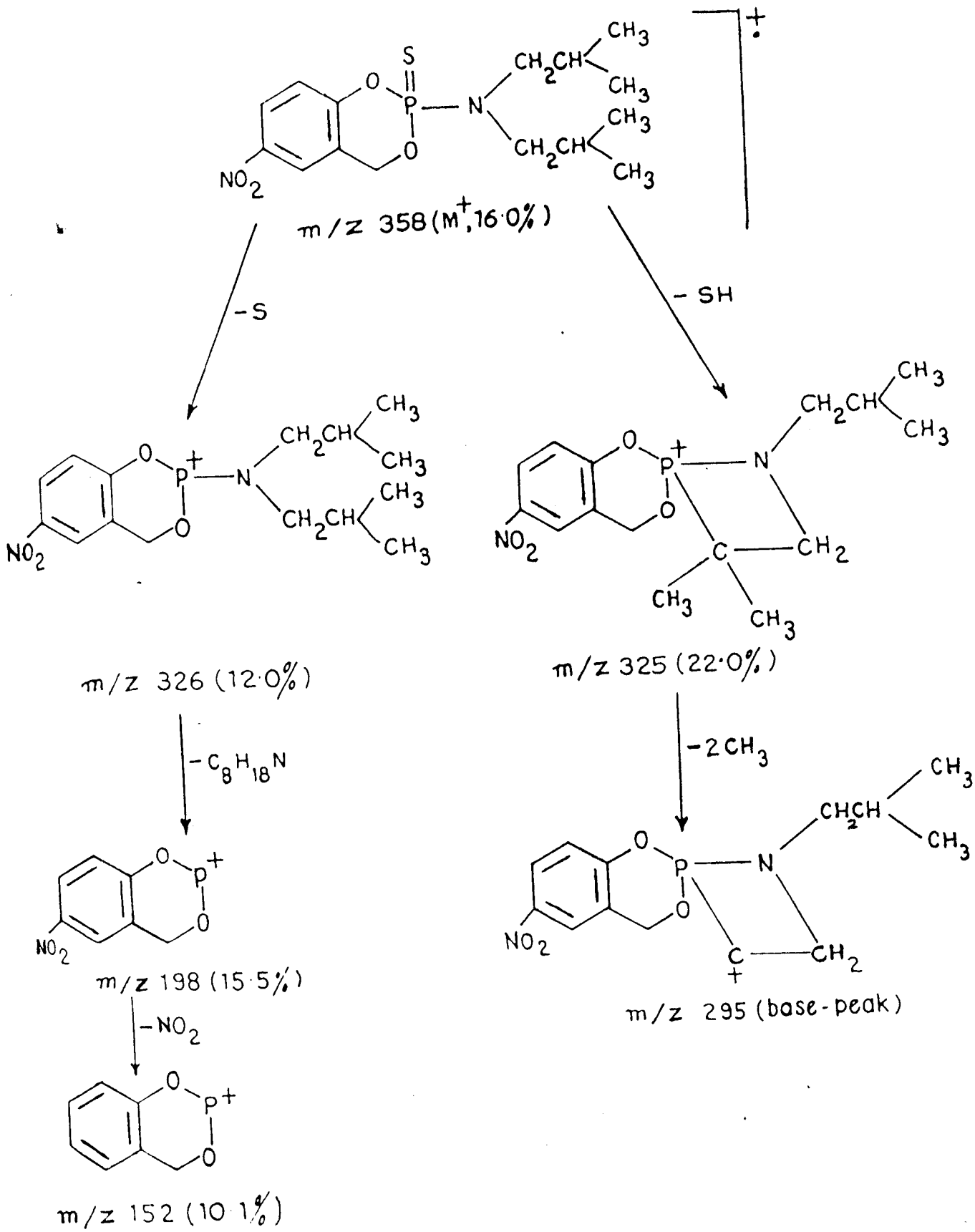
Mass fragmentation of BR-27 (Scheme -7)



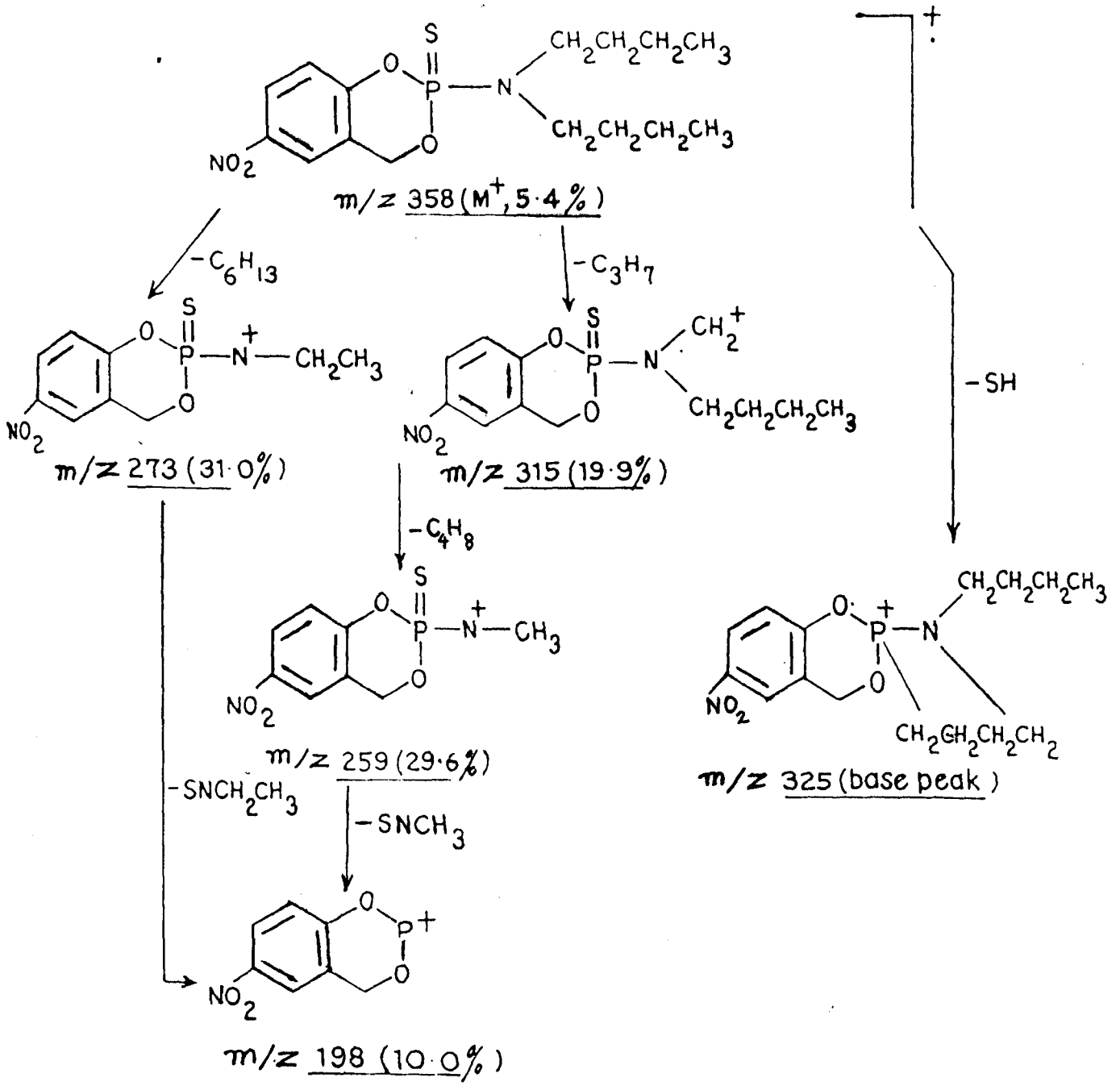
Mass fragmentation of BD-1 (Scheme - 8)



Mass fragmentation process of BD-5 (Scheme - 9)



Mass fragmentation of BD-25 (Scheme -10)



MASS FRAGMENTATION OF BD-29 (Scheme-11)

BR-6 (Scheme - 4)

This diisobutylamido compound (BR-6) shows m/z 348 ion as the base peak by the elimination of C_3H_7 from the molecular ion (m/z 391, % RI 15). The ion (m/z 358, % RI 20) is formed by the direct elimination of SH from the molecular ion. The ion (m/z 174, % RI 15) is formed by the loss of C_7H_5BrO from the ion, m/z 358. The ion (m/z 292, % RI 85) is formed by the loss of C_4H_8 from the ion, m/z 348. The ion (m/z 57, % RI 62.5) is formed by the elimination of $C_{11}H_{14}NC_2PSBr$ from the molecular ion, m/z 391. The ion (m/z 43, % RI 40) is formed from the molecular ion, m/z 391 by the loss of $C_{12}H_{16}NO_2PSBr$. The molecular ion m/z 391, by the elimination of $C_8H_{18}N$ forms the ion (m/z 263, % RI 20). The ion (m/z 231, % RI 22.5) is formed from the ion, m/z 263, by the elimination of 'S' radical.

BR-10 (Scheme - 5)

In the spectrum of dibutylamido compound (BR-10), the base peak is due to m/z 358 ion (Scheme-5) formed by the elimination of SH from the molecular ion (m/z 391, % RI 57.5). The ion (m/z 348, % RI 22.5) is formed by the elimination of C_3H_7 from the molecular ion. The ion (m/z 292, % RI 16.25) is formed by the elimination of C_4H_8 from the ion, m/z 348. The ion (m/z 305, % RI 22.5) is formed by the loss of C_3H_7 from the ion, m/z 348. The ion (m/z 174, % RI 50) is formed by the loss of C_7H_5BrO from the ion, m/z 358. The ion (m/z 57, % RI 17.5) is formed by the elimination of $C_{11}H_{14}NO_2PSBr$ from the molecular ion. The ions (m/z 263, % RI 10) and (m/z 231, % RI 23.75) have also been observed.

BR-24 (Scheme - 6)

This compound shows m/z 302 ion as the base peak by the direct elimination of SH from the M^+ ion peak (m/z 335, % RI 35.5). The ion (m/z 184, % RI 21.5) and the ion (m/z 231, % RI 31.1) are formed by the direct loss of C_4H_9NOP and C_4H_9N respectively from the base peak ion. The ion (m/z 156, % RI 15.3) is formed by the loss of CO.

BR-27 (Scheme - 7)

In the spectrum of N-hexyl amido compound (BR-27), the ion (m/z 330, % RI 2) is formed by the elimination of SH from the molecular ion (m/z 363, % RI 1) (Scheme -7). The base peak is due to m/z 184 ion, formed by the elimination of $C_6H_{14}NOPS$ from the molecular ion (m/z 363, % RI 1). The ion (m/z 156, % RI 49.0) is formed by the elimination of CO from the ion, m/z 184. The ion (m/z 77, % RI 97) is formed by the elimination of Br from the ion (m/z 156, % RI 49.0).

BD-1 (Scheme-8)

The most interesting mass fragmentation processes of the compound (BD-1) show the β-cleavage (m/e 59, % RI 26) accompanied by the elimination of $-CH_2$ and -H leading to the formation of the peaks at (m/e 45, % RI 12) and the base peak at m/e 58 (% RI 100) respectively. The base peak does not contain the phosphorus moiety.

BD-5 (Scheme - 9)

The β-cleavage at the exocyclic ester group of the isopropoxy compound with and without single hydrogen rearrangement leads to the formation of the peak (m/z 247, % RI 56.0) and

the base peak (m/z 247, % RI 56.0) and the base peak (m/z 246) respectively, the peak (m/z 214, % RI 72.0) is observed owing to the elimination of SH from the peak at m/z 247 and -S from the peak at (m/z 246). A further elimination of -O from the peak at (m/z 214) gives the peak (m/z 198, % RI 4.0).

BD-9

M^+ - 323 (Base peak)

BD-25 (Scheme - 10)

Diisobutylamido compound shows (m/z 295) as the base peak. Direct elimination of SH from the molecular ion peak (m/z 358, % RI 16.0) resulted in the formation of the ion (m/z 325, % RI 22.0). The ion (m/z 326, % RI 12.0) is formed by loss of S from the molecular ion peak, elimination of NC_8H_{18} from it results the formation of the ion (m/z 198, % RI 15.5) and then the ion (m/z 152, % RI 10.1) is obtained by the loss of NO_2.

BD-29 (Scheme - 11)

Dibutylamido compound shows base peak ion (m/z 325) by the direct loss of SH from the molecular ion peak (m/z 358, % RI 5.4). Ions (m/z 315, % RI 19.9) and (m/z 273, % RI 31.0) are obtained from the molecular ion peak by the elimination of C_3H_7 and C_6H_{13} respectively. Ion (m/z 198, % RI 10.0) is also observed.

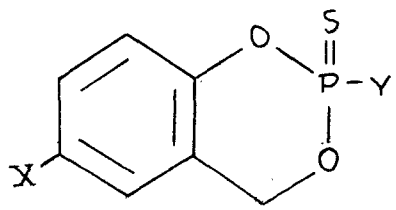
3. Insecticidal activity:

3.(a) Insecticidal activity on Cockroaches

The oral insecticidal data on Cockroaches (P. americana) for different compounds including salithion have been tabulated in Table - 3.

Table - 3

Oral Insecticidal activity on Cockroaches (*P. americana*)



where, X = Cl, Br, NO_2

Y = alkylamido group, alkoxy group

Code No.	X	Y	Conc. showing 100% mortality (LC_{100}/µg/gm)
CL-6	Cl	N,N-Diisobutylamido	> 50
CL-10	Cl	N,N-Dibutylamido	> 50
CL-24	Cl	N-Butylamido	10
BR-6	Br	N,N-Diisobutylamido	> 50
BR-10	Br	N,N-Dibutylamido	> 50
BR-24	Br	N-Butylamido	> 50
BR-27	Br	N-Hexylamido	> 50
BD-1	NO_2	Methoxy-ethoxy	35-50
BD-5	NO_2	Isopropoxy	15
BD-9	NO_2	Phenoxy	> 50
BD-25	NO_2	N,N-Diisobutylamido	> 50
BD-29	NO_2	N,N-Dibutylamido	> 50
BD-8	NO_2	Methoxy	6
Salithion		Methoxy	10

The data reveals that all the chloro/bromo/nitro saligenin cyclic phosphoramidothionates have less oral insecticidal activity than BD-8[18] (2-Methoxy-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-sulphide) and salithion. The methoxy compound (BD-8) is most active and its insecticidal activity on Cockroach is about 1.5 times greater than that of salithion. Salithion has about 1.5 times greater insecticidal activity than that of the

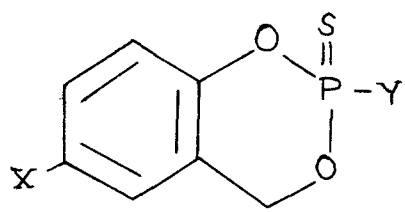
isopropoxy compound (BD-5), 3.5-5.0 times greater than methoxy-ethoxy compound (BD-1) but in case of chloro derivative of N-butylamido compound (CL-24) the insecticidal activity is almost similar to that of salithion. The other compounds have less insecticidal activity.

3. (b) Insecticidal activity (contact toxicity) on Blow-fly (C. megacephala)

The contact toxicity data on Blow-fly (C. megacephala) have been presented in Table - 4.

Table - 4

Contact toxicity on Blow-fly (C. megacephala)



Code No.	X	Y	LD_{50} µg/Blow-fly (female)
CL-6	Cl	N,N-Diisobutylamido	> 10
CL-10	Cl	N,N-Dibutylamido	> 10
CL-24	Cl	N-Butylamido	> 10
BR-6	Br	N,N-Diisobutylamido	> 10
BR-10	Br	N,N-Dibutylamido	> 10
BR-24	Br	N-Butylamido	> 10
BR-27	Br	N-Hexylamido	> 10
BD-1	NO_2	Methoxy-ethoxy	> 10
BD-5	NO_2	Isopropoxy	2
BD-9	NO_2	Phenoxy	> 10
BD-25	NO_2	N,N-Diisobutylamido	> 10
BD-29	NO_2	N,N-Dibutylamido	> 10
BD-8	NO_2	Methoxy	0.3
Salithion		Methoxy	0.5

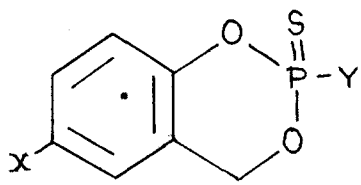
From the table we find that none of the chloro/bromo/nitro compounds possess any contact insecticidal activity on Blow-flies except isoporpoxy (BD-5) and methoxy (BD-8) compound. The toxicity of iso-propoxy compound (BD-5) is less than that of salithion, but the methoxy compound (BD-8) is more toxic than salithion.

3.(c) Insecticidal activity of Grasshoper (O. nitidula)

The insecticidal activity data is listed in Table - 5, against Grasshopper (O. nitidula).

Table - 5

Insecticidal activity on Grasshopper (O. nitidula)



Code No.	X	Y	Average LC_{100} value ($\mu g/gm$)
CL-6	Cl	N,N-Diisobutylamido	> 10
CL-10	Cl	N,N-Dibutylamido	> 10
CL-24	Cl	N-Butylamido	> 10
BR-6	Br	N,N-Diisobutylamido	> 10
BR-10	Br	N,N-Dibutylamido	> 10
BR-24	Br	N-Butylamido	> 10
BR-27	Br	N-Hexylamido	> 10
BD-1	NO_2	Methoxy-ethoxy	3.5
BD-5	NO_2	Isopropoxy	1.5
BD-9	NO_2	Phenoxy	> 10
BD-25	NO_2	N,N-Diisobutylamido	> 10
BD-29	NO_2	N,N-Dibutylamido	> 10
BD-8	NO_2	Methoxy	0.4
Salithion		Methoxy	0.5

The table shows that the methoxy compound (BD-8) is most active, and its insecticidal activity is comparable with that of salithion, BD-5 and BD-1 have some insecticidal activity but less than that of salithion. The other compounds are non-insecticidal.

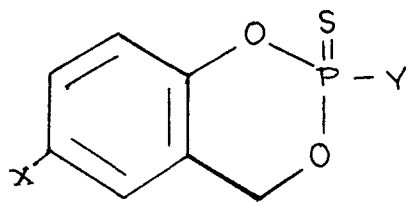
Eto et al[32] reported that the methoxy compound has about 60 times less insecticidal activity as compared to salithion. We however observed that, in case of Cockroaches, the insecticidal activity is greater than that of salithion, and in case of Grasshoppers, the insecticidal activity is comparable with that of salithion. However, Eto et al obtained the methoxy compound in paste form which may be an impure one.

3.(d) Insecticidal activity on Aphid (Lipaphis erysimi)

The data reveals (Table - 6) that, all the chloro/bromo/nitro saligenin cyclic phosphoramidothionates have less insecticidal activity than BD-8[18]. The ED_{95} value at 48 hours against Aphids increases in the order BD-8 < CL-6 < CL-10 < BD-29 < BD-1 < BR-6 < BD-5 < BR-27 < BD-25 < BR-24 < BR-10 < CL-24 < BD-9. Therefore the Phenoxy compound BD-9 has the least insecticidal activity. The data was corrected according to Abbott's formula[35].

Table - 6

Insecticidal activity on Aphid (*Lipaphis erysimi*)

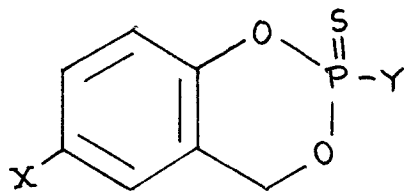


Code No.	X	Y	ED_{95} (μg/ml solution) on 100 Aphids (after 48 hours)
CL-6	Cl	N,N-Diisobutylamido	10.5
CL-10	Cl	N,N-Dibutylamido	10.5
CL-24	Cl	N-Butylamido	15.0
BR-6	Br	N,N-Diisobutylamido	12.6
BR-10	Br	N,N-Dibutylamido	14.6
BR-24	Br	N-Butylamido	13.3
BR-27	Br	N-Hexylamido	13.2
BD-1	NO_2	Methoxy-ethoxy	11.9
BD-5	NO_2	Isopropoxy	12.9
BD-9	NO_2	Phenoxy	20.8
BD-25	NO_2	N,N-Diisobutylamido	13.2
BD-29	NO_2	N,N-Dibutylamido	11.2
BD-8	NO_2	Methoxy	9.7

4. Acute Oral toxicity on Rat:

Table - 7

Acute Oral toxicity of different compounds on Rat



Where, X = Cl, Br, NO_2

Y = alkylamido group, alkoxy group.

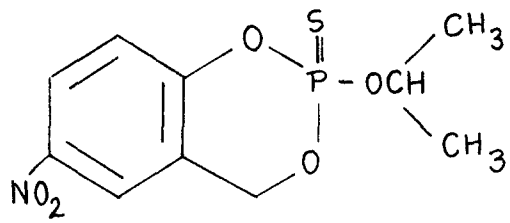
Code No.	X	Y	LD_{50} (mg/kg)
CL-6	Cl	N,N-Diisobutylamido	> 250
CL-10	Cl	N,N-Dibutylamido	> 250
CL-24	Cl	N-Butylamido	> 250
BR-6	Br	N,N-Diisobutylamido	> 250
BR-10	Br	N,N-Dibutylamido	> 250
BR-24	Br	N-Butylamido	> 250
BR-27	Br	N-Hexylamido	> 250
BD-1	NO_2	Methoxy ethoxy	> 250
BD-5	NO_2	Isopropoxy	140
BD-9	NO_2	Phenoxy	> 250
BD-25	NO_2	N,N-Diisobutylamido	> 250
BD-29	NO_2	N,N-Dibutylamido	> 250
BD-8	NO_2	Methoxy	130
Salithion		Methoxy	102

The acute oral toxicity (LD_{50}) of different compounds were investigated on male white albino rat, which have been tabulated in Table - 7 and the results have been compared with salithion (LD_{50} value for salithion has been taken from Eto et al[33]). The toxic symptoms and mortality caused by single administration of the methoxy compound (BD-8) and the isopropoxy compound (BD-5) are showed in Table - 8 and Table - 9 respectively.

All compounds are less toxic than that of salithion; the methoxy compound (BD-8) and the isopropoxy compound (BD-5) have greater toxicity compared to other nitro/chiloro/bromo saligenin cyclic phosphoramidothionates. The compounds BR-10, BR-24, CL-6 and BR-27 showed some toxic symptoms, which are given in detail in Table-10,11,12,13. In all cases,decrease in spontaneous motor activity was observed. After 2-3 hours for compounds (BR-10, BR-24, CL-6 and BR-27) the rats suffered from irregular respiration, salivation and weakness in activity.

Table - 8

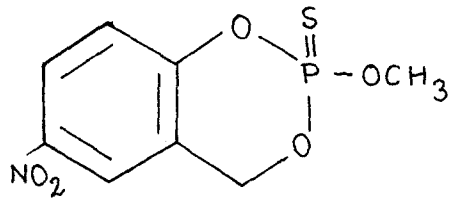
Acute oral toxicity of the Iso-propoxy compound (BD-5) on Rats



Dosage (mg/kg)	Daily mortality 1	2	10	Mortality (percent)	Symptoms	LD_{50} (mg/kg)
50	0/2	0/2	0/2	0	-	-
100	0/2	0/2	0/2	0	-	-
120	0/4	1/4	1/4	25	-	-
140	0/6	3/6	3/6	50	Decrease of spontaneous motor activity after 1-2 hours, anorexia, recovered to normal after 2-3 days.	140
160	3/6	6/6	6/6	100	Decrease of spontaneous motor activity after 1 hour; motor ataxia after 2-3 hours; motor ataxia became severe; irregular respiration, salivation.	

Table - 9

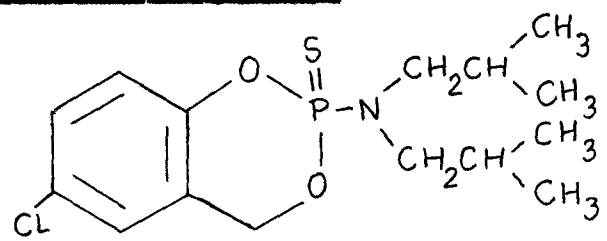
Acute oral toxicity of the Methoxy compound (BD-8) on Rat



Dosage (mg/kg)	Daily mortality 1	2	10	Mortality (percent)	Symptoms	LD_{50} (mg/kg)
100	0/2	0/2	0/2	0	Decrease of spontaneous motor activity after 2-3 hours; recovered to normal in 2-3 days.	-
130	0/6	3/6	3/6	50	Decrease of spontaneous motor activity after 1 hour; motor ataxia after 3 hours; salivation and irregular respiration, motor ataxia become severe; recovered to normal in 4-5 days.	130
160	2/6	6/6	6/6	100	Decrease of spontaneous motor activity within 1 hour; the other symptoms are same as above.	-

Table - 10

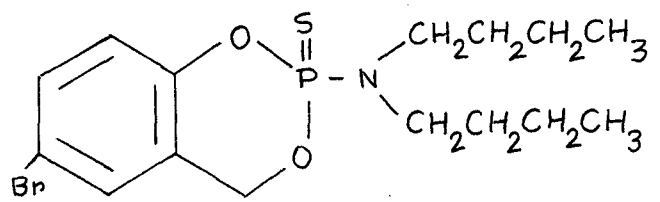
Acute oral toxicity of the N,N-diisobutylamido compound (CL-6) on Rats



Dosage (mg/kg)	Daily mortality 1	2	10	Mortality (percent)	Symptoms	LD_{50} (mg/kg)
100	0/2	0/2	0/2	0	-	-
200	0/6	0/6	0/6	0	slight weakness in movement.	-
250	0/6	0/6	0/6	0	Hair falling was observed but it recoveed. Weakness in movement, no other symptoms.	-

Table - 11

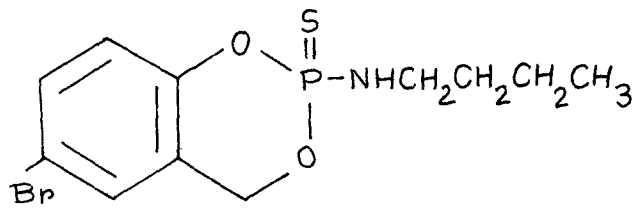
Acute oral toxicity of the N,N-dibutylamido compound (BR-10) on Rats



Dosage (mg/kg)	Daily mortality 1	2	10	Mortality (percent)	Symptoms	LD_{50} (mg/kg
100	0/2	0/2	0/2	0	Sligh weakness.	-
200	0/6	0/6	0/6	0	Weakness in walking.	-
250	0/6	0/6	0/6	0	Weakness in motor activity and during walking staggering movement was found.	-

Table - 12

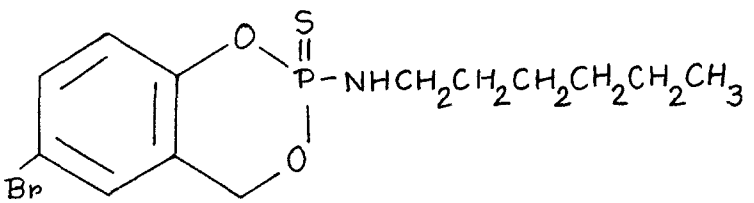
Acute oral toxicity of the N-butylamido compound (BR-24) on Rats



Dosage (mg/kg)	Daily mortality 1	2	10	Mortality (Percent)	Symptoms	LD_{50} (mg/kg)
100	0/2	0/2	0/2	0	Weakness, but recovered soon.	-
200	0/6	0/6	0/6	0	Weakness, recovered.	-
250	0/6	0/6	0/6	0	Weakness, lesions are found in the body, trouble in walking, not taking normal diet.	-

Table - 13

Acute oral toxicity of the N-hexyl amido compound (BR - 27) on Rats



Dosage (mg/kg	Daily mortality			Mortality (percent)	Symptoms	LD_{50} (mg/kg)
	1	2	10			
100	0/2	0/2	0/2	0	Normal.	-
200	0/6	0/6	0/6	0	Normal.	-
250	0/6	0/6	0/6	0	Weakness in movement, no other symptoms.	-

5. Histopathological study of some organs, of the Rat treated with the compound Cl-6

Histopathological study of some organs of rat, on application of compound Cl-6 (Diisobutylamido) were carried out. Observations are given below. The results were compared with their respective controls.

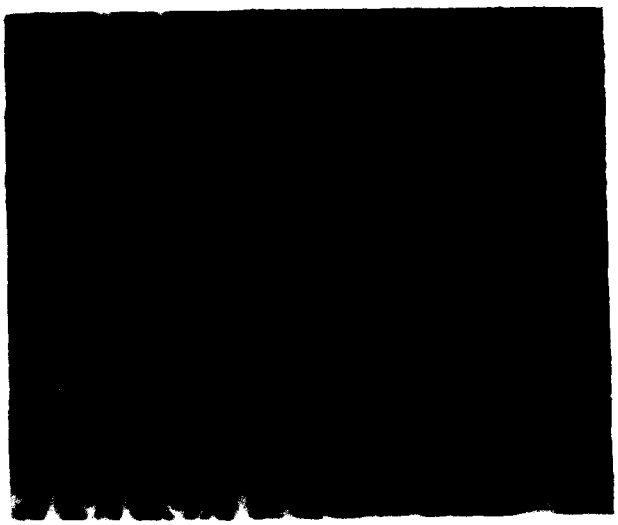
Kidney:

The normal kidney (control) section of rat showed the assemblage of tubular structures of various sizes. Convoluted blood capillaries forming a few compact glomerular mass of renal corpuscles were also observed. Tubular structures were the uriniferous tubules, among which were smaller neck segments bearing cilia towards the lumen, proximal segment having larger columnar epithelial cells with brush border, that further led to distal segment with darkly stained cells and collecting duct of the uriniferous tubules (Fig. a).

The Kidney section of the rat, which was treated with the compound showed that, some of the uriniferous tubules were mildly dialated and their walls ruptured. Some cells of the tubules were enlarged. Some renal tubules were enlarged and their epithelial linings were also ruptured. Deeply stained clump of cells were found. A number of Glomerulus were disorganised and Bowman's capsules appeared to be normal (Fig. b,c).

Liver:

A section of untreated liver (control) showed continuous mass of roughly hexagonal hepatic cells with comparatively large central nucleus. Hepatic cells were arrange in cords, each cord

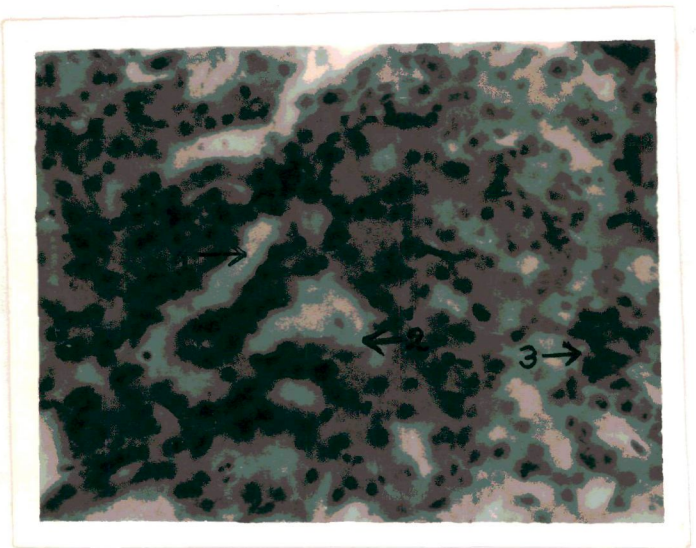


(a)

(b)

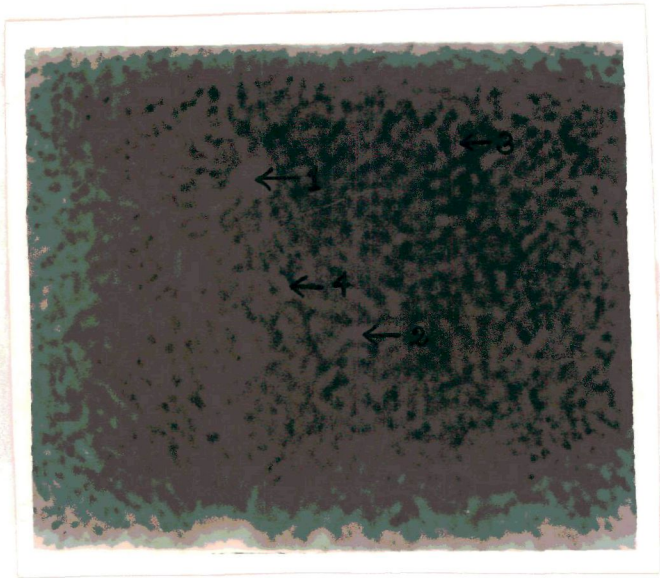
Figure - (a): Microphotograph of the section of untreated Kidney of rat. Normal structure X 450.

Figure - (b): Microphotograph of the section of treated (compound) Kidney of rat shows, (1) Bowman's capsule reduced. (2) elongated renal tubule with thin epithelial lining. Normal structure X 450.

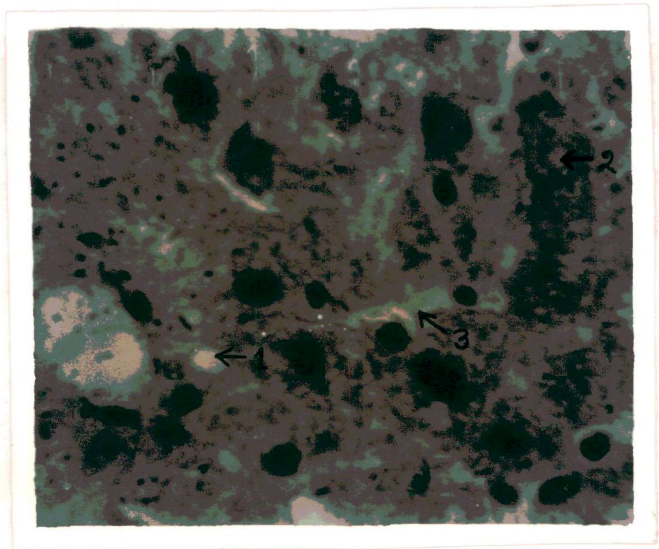


(c)

Figure - (c): Microphotograph of the section of treated (compound) kidney of rat shows, (1) lumen of renal tubule elongated, (2) destroyed epithelial lining of renal tubule, (3) deeply stained clump of cells. Normal structure X 450



(d)



(e)

Figure - (d): Microphotograph of the section of untreated Liver of rat. Normal structure X 100.

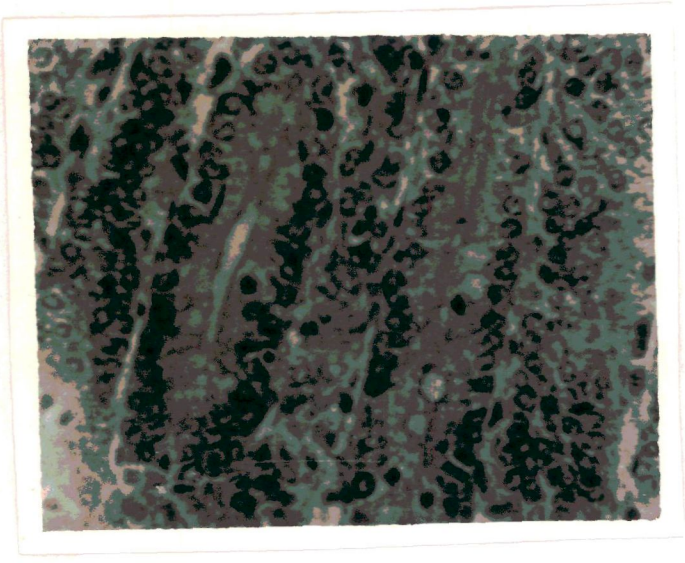
Figure - (e): Microphotograph of the section of treated (compound) liver of rat shows, (1) vacuoles in the hepatic cells, (2) hepatic cells without nuclei, (3) ruptured cell membrane. Normal structure X 1000.

being separated by a blood space lined by connective tissue. Large blood sinusoids filled with blood were seen, from which numerous blood capillaries emerged and transversed in to the hepatic mass. Thin bile cannaliculae were abundant between the hepatic cells (Fig. d).

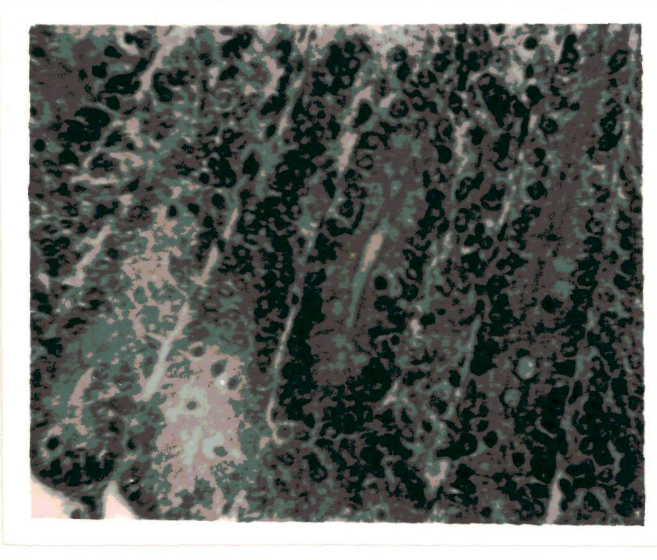
The liver section of the rat, which was treated with the compound showed that in some of the hepatic cells vacuoles were formed, and the cell membranes were ruptured. Some of the hepatic cells were without nuclei. Cytoplasm of some cells were precipitated; but the other characters were normal like control (Fig. e).

Intestine:

The sections of intestine of the rat which were untreated (control) and treated with the compound, showed the same characters. They showed the usual four layered structures. The outer most serosa was followed by a muscular coat, consisting of an outer longitudinal and an inner circular layer. Submucosa was divided into an outer stratum compactum, a dense connective tissue arranged in a wavy pattern and an inner stratum granulosum rich in capillary net work. The latter merged with the tunica propria of the underlying mucosal coat, there being no muscular mucosa. The epithelial lining of the mucosa consisted of prismatic cells with basal nuclei. The nuclei of the intestinal mucosal cells were round with 2 to 3 nucleoli. The intestinal mucosal cells showed serrated margin with a number of interspaced goblet cells. The mucosal layer was thrown in to folds (Fig. f,g)



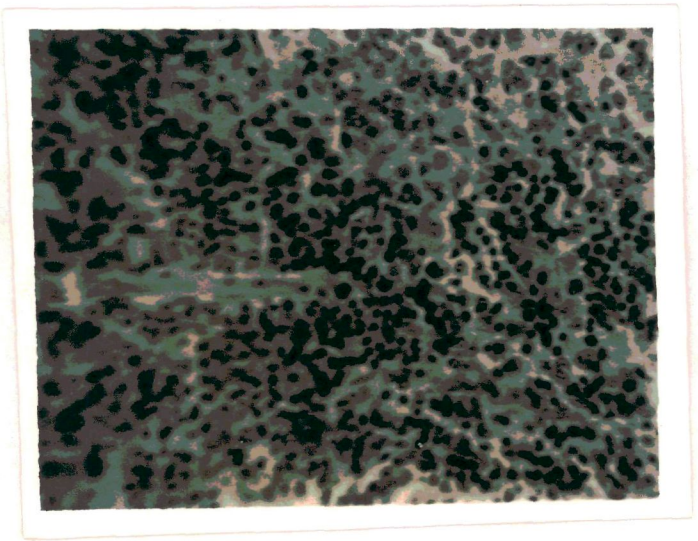
(f)



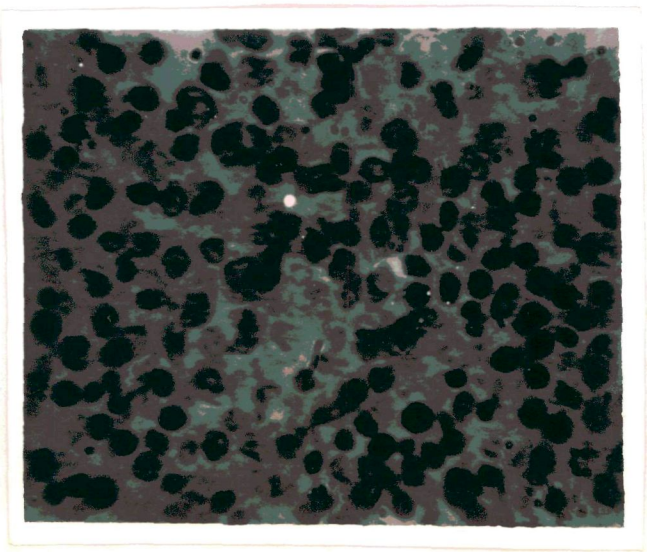
(g)

Figure - (f): Microphotograph of the section of untreated intestine of rat. Normal structure X 450.

Figure - (g): Microphotograph of the section of treated (compound) intestine of rat shows no abnormality. Normal structure X 450.



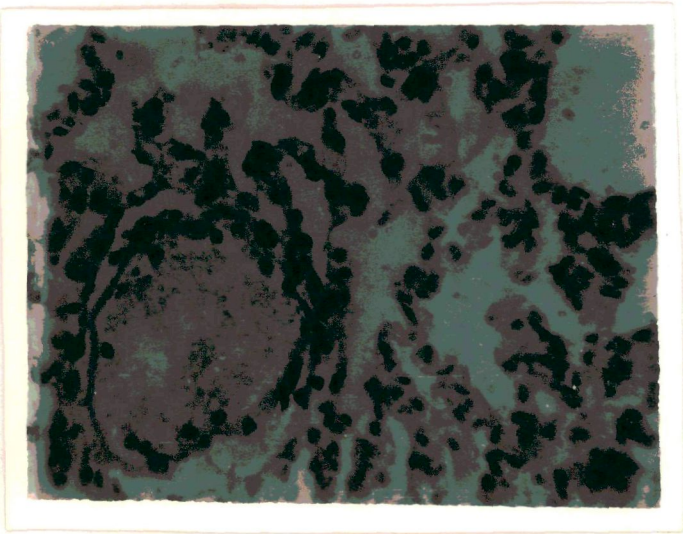
(h)



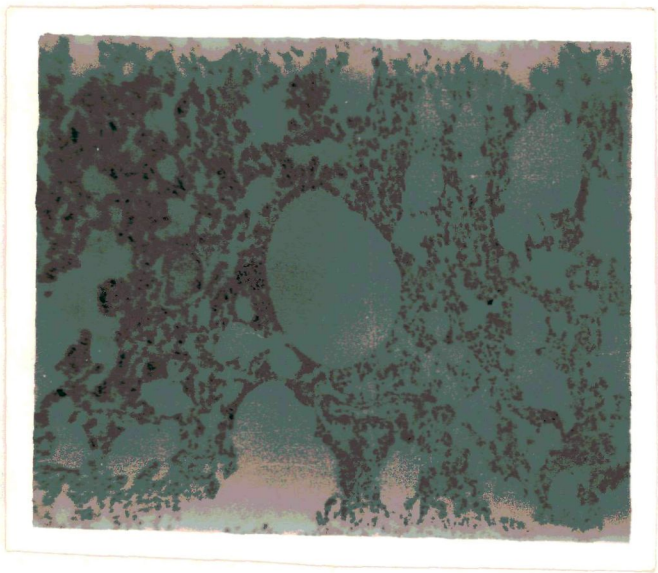
(i)

Figure - (h): Microphotograph of the section of untreated spleen of rat. Normal structure X 1000.

Figure - (i): Microphotograph of the section of treated (compound) spleen of rat shows no abnormality. Normal structure X 1000.



(j)



(k)

Figure - (j): Microphotograph of the section of untreated lung of rat. Normal structure X 100.

Figure - (k): Microphotograph of the section of treated (compound) lung of rat shows no abnormality. Normal structure X 100.

Lung:

The sections of lung untreated (control) and treated with the compound showed no difference in histological characters. Numerous alveoli were present and the alveoli were formed into clusters, which opened in an alveolar duct. Each bronchus as it entered into the lungs, divided and sub-divided into finer branches the bronchioles. The bronchioles were sub-divided into respiratory bronchioles. The respiratory bronchiole gave rise to several alveolar ducts which opened into the alveoli (Fig. h, i)

Spleen:

Both the sections, which were untreated (control) and treated with the compound showed, no differences in characters. For both of them, the spleen was surrounded by a thin capsule made up of fibrous connective tissue and involuntary muscles. The trabaculae contained the fibrous connective tissue. The spleen was composed of lymphatic tissues which could be distinguished into white and red pulp. The white pulp consisted of reticular fibres and formed a seath around the arteries (Fig. j, k)

6. Acute Oral Toxicity and Delayed Neurotoxicity in Hens:

(a) Acute Oral Toxicity:

The toxic symptoms and mortality caused by single administration of each compound are reproduced in Table-15, 16, 17 and 18. The LD_{50} values are tabulated below(Table-14)

Table -14

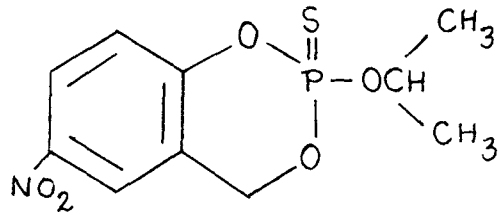
Code No.	LD_{50} (mg/kg)
CL-6	> 200
BR-6	> 300
BD-5	160
BD-8	100
-	110

The LD_{50} value of salithion has been taken from Kadota et al[34].

The methoxy compound is most toxic. By single oral administration of 100 mg/kg of the methoxy compound, decrease of spontaneous motor activity, salivation, motor ataxia became severe, irregular respiration was observed, and three out of six hens died within 48 hours and the rest recovered to normal state in 4-5 days. The Isopropoxy (BD-5); N,N-Diisobutylamido (CL-6); N,N-Diisobutylamido (BR-6) compounds are less toxic as compared to salithion and their toxic symptoms are given in the Tables (Tables 15 to 17).

Table - 15

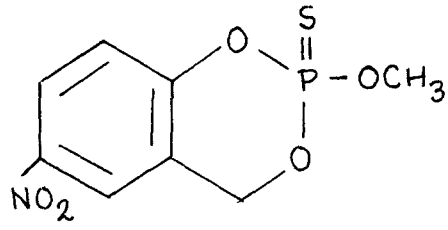
Acute Oral Toxicity of the Isopropoxy compound (BD-5) in Hens.



Dosage mg/kg	Daily Mortality 1	2	3	Mortality (percent)	Symptoms	LD_{50} (mg/kg)
100	0/2	0/2	0/2	0	Decrease of spontaneous motor activity after 2-3 hours, recovered to normal within 2 days.	-
160	0/6	3/6	3/6	50	Decrease of spontaneous motor activity after 1-2 hours; salivation, irregular respiration; motor ataxia became severe; recovered to normal in 5-6 days.	160
200	3/6	6/6	6/6	100	Same as above.	-

Table - 16

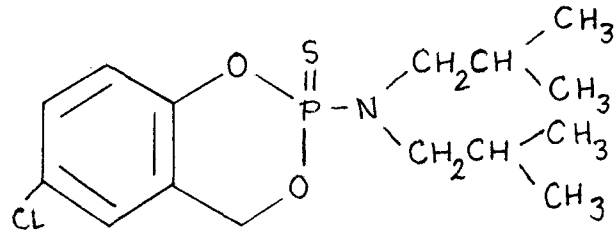
Acute Oral Toxicity of the Methoxy Compound (BD-8) in Hens



Dosage (mg/kg)	Daily Mortality			Mortality (percent)	Symptoms	LD_{50} (mg/kg)
	1	2	10			
50	0/6	0/6	0/6	0	Decrease of spontaneous motor activity after 2-3 hours, recovered to normal within 2 days.	-
100	1/6	3/6	3/6	50	Decrease of spontaneous motor activity after 1-2 hours; salivation, irregular respiration; motor ataxia become severe; recovered to normal in 4-5 days.	100
150	3/6	5/6	6/6	100	Same as above	-

Table - 17

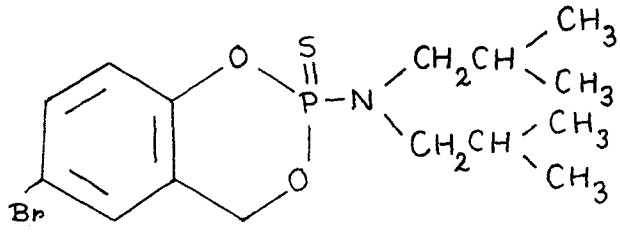
Acute Oral Toxicity of the N,N, Diisobutylamido compound (CL-6) in Hens



Dosage (mg/kg)	Daily mortality			Mortality (percent)	Symptoms	LD_{50} (mg/kg)
	1	2	10			
100	0/5	0/5	0/5	0	Falling of feathers, decrease of spontaneous motor activity.	-
150	0/5	1/5	1/5	20	Falling of feather, decrease of spontaneous motor activity, irregular breathing.	-
200	1/5	2/5	2/5	40	Falling of feathers, decrease of spontaneous motor activity.	> 200

Table - 18

Acute Oral Toxicity of the N,N, Diisobutylamido compound (BR-6) in Hens



Dosage (mg/kg)	Daily Mortality			Mortality (percent)	Symptoms	LD_{50} (mg/kg)
	1	2	10			
100	0/5	0/5	0/5	0	Normal	-
200	0/5	0/5	0/5	0	Normal	-
300	0/5	0/5	0/5	0	Decrease of spontaneous motor activity. Trembling during walking.	-

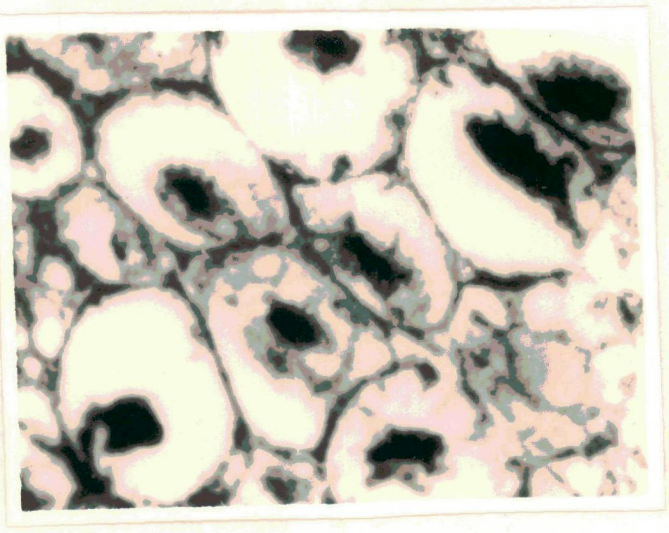
(b) Delayed Neurotoxicity:

Permanent paralysis in legs of hen were observed after applying the N,N-Diisobutylamido (CL-6) compound. It was observed that, after six months of application of twice the dose of 100 mg/kg (dose interval was one month), after five months of application of single dose of 200 mg/kg, after three months of application of single dose of 250 mg/kg, there was paralysis of the legs. However there was no neurotoxic symptoms upto six months after the application of single dose of 100 mg/kg. After histopathological examinations and demyelimination of sciatic nerves were noted (Fig. A, B)

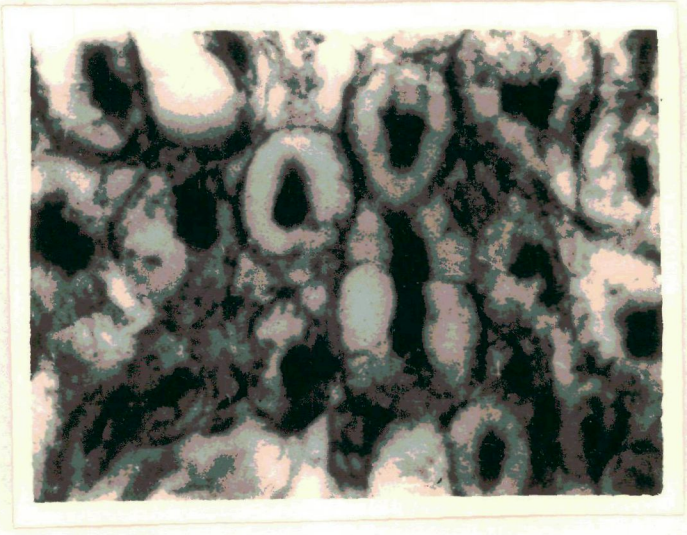
The experiment was also performed in other three compounds viz. N, N-Diisobutylamido (BR-6), Isoporpoxy (BD-5) and Methoxy (BD-8) compounds. In BR-6 the doses applied were 100 mg/kg (single dose), 100 mg/kg (the dose applied twice) and 200 mg/kg (single dose). In BD-5 the doses were 100 mg/kg (single dose) and 100 mg/kg (the dose applied twice). In BD-8 the does were 50 mg/kg (single dose) and 50 mg/kg (the dose applied twice). During the whole observation period of hens treated with compounds BR-6, BD-5 and BD-8, showed no paralysis in legs and histopathological findings of sciatic nerves were normal.

All the above observations were tabulated in summarized form (Table -19).

Nerve
F Hen.



(A)



(B)

Figure: Oil immersion photograph of sciatic nerve in cross section after standard histological preparation and Haematoxylin and Eosin stain X 4000. (A) - Control hen. (B) - Treated hen

Table - 19

Delayed Neurotoxic action of four compounds on Hens

Compound	Code No.	Duplicate administration Dose (mg/kg)	Mortality	Paralysis	Remarks
N,N-Diisobutylamido	CL-6	100x1	0/4	No	No administration of Atropine sulphate
		100x2	0/4	Paralysis	
		200x1	0/4	"	Atropine sulphate was administered hourly
		250x1	0/4	"	
N,N-Diisobutylamido	BR-6	100x1	0/4	No	
		100x2	0/4	"	No administration of Atropine sulphate
		200x1	0/4	"	
Isopropoxy	BD-5	100x1	0/4	No	"
		100x2	0/4	"	
Methoxy	BD-8	50x1	0/4	No	
		50x2	0/4	"	"

7. Anticholinesterase Activity:

The acetylcholinesterase inhibition data of the compounds for Blow-fly head homogenate (BFAChE) and Goat whole blood (AChE) are listed in Appendix, Experiment No. 14 to 25 (pp. 247 to 258), 26 to 37 (pp 259 to 270) respectively. The molar I_{50} values calculated by least square regression programme are given in Table - 20.

The molar I_{50} value of the 6-Nitro/chloro/bromo saligenin cyclic phosphoramidothionates for BFAChE increases in the following order:

BD-1 < BD-9 < BD-5 < BD-29 < BD-25 < BR-27 < BR-24 < CL-24 < BR-10 < CL-10 < BR-6 < CL-6

i.e. the compound BD-1 has the highest acetylcholinesterase inhibitory activity for BFAChE.

For Goat whole blood (AChE), the I_{50} value increases in the order:

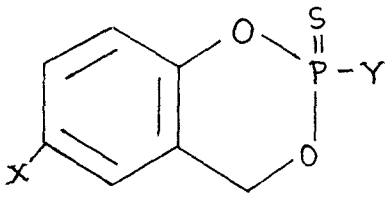
BD-25 < BD-29 < BD-5 < BD-9 < BR-10 < BD-1 < CL-10 < CL-6 < BR-27 < BR-24 < CL-24 < BR-6.

The anticholinesterase activity of BD-25 is highest.

From the above results, it reveals that the nitro compounds (BD-1 and BD-25) show more inhibitory activity on goat whole blood and blow-fly head homogenate than the chloro and bromo compounds.

Table - 20

Anticholinesterase activity of different compounds against Blow-fly head homogenate (BFAChE) and Goat whole blood (AChE)



Where, X = Cl, Br, NO_2
Y = alkylamido group
alkoxy group

Code No.	X	Y	I_{50} (BFAChE) (M) x 10^3	I_{50} (AChE) (M) x 10^3
CL-6	Cl	N,N-Diisobutylamido	80.38	4.25
CL-10	Cl	N,N-Dibutylamido	9.40	4.13
CL-24	Cl	N-Butylamido	0.3115	5.996
BR-6	Br	N,N-Diisobutylamido	9.84	28.93
BR-10	Br	N,N-Dibutylamido	3.15	1.22
BR-24	Br	N-Butylamido	0.04397	4.554
BR-27	Br	N-Hexylamido	0.04827	4.478
BD-1	NO_2	Methoxy-ethoxy	0.0005112	4.09
BD-5	NO_2	Isopropoxy	0.0025	0.1072
BD-9	NO_2	Phenoxy	0.001337	1.15
BD-25	NO_2	N,N-Diisobutylamido	0.0276	0.01409
BD-29	NO_2	N,N-Dibutylamido	0.00948	0.0416

8. Antifungal activity:

The fungicidal activity of some 2-alkylamido-6-nitro/chloro/bromo-4H-1,3,2-benzodioxaphosphorin-2-sulphide were tested in vitro against Helminthosporium oryzae and Pyricularia oryzae. The data (percentage inhibition) for growth inhibition studies against H. oryzae and P. oryzae are listed in Appendix, Experiment No.38-49 (pp 271 to 282) and Expt. No.52-61 (pp 283 to 294) respectively. The ED_{50} and ED_{95} values (μg/ml) were calculated by least square regression programme and presented in Table - 21 and Table - 22 respectively. The data for Edifenphos (Hinosan) as a standard have also been presented for comparison. Thus the ED_{50} values at 72 hours against H. oryzae increase in the following order:

CL-10 < BR-10 < BR-6 < CL-6 < CL-24 < BR-24 < BR-27 < BD-29 < BD-25

The ED_{95} values at 72 hours against H. oryzee increase in the order:

CL-10 < CL-6 < BR-6 < BR-10 < CL-24 < BR-24 < BD-29 < BD-25 < BR-27.

The ED_{50} values at 72 hours against P. oryzae increase in the order:

BR-6 < CL-10 < CL-6 < BR-10 < CL-24 < BR-27 < BR-24 < BD-25 < BD-29.

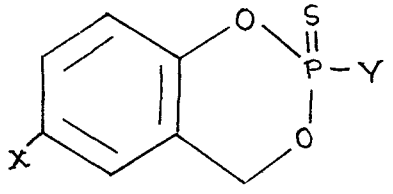
The ED_{95} values at 72 hours against P. oryzae increase in the order:

BR-6 < CL-10 < CL-6 < BR-10 < CL-24 < BR-24 < BD-29 < BR-27 < BD-25.

A comparative study shows that at 72 hours, the antifungal activity for both the fungi is lowest for BD-25 i.e. the Diisobutylamido compound. The effects of chloro and bromo compounds are almost comparable to Edifenphos (Hinosan) in case of P. oryzae. For H. oryzae the activities are 10-80 times greater than that of Edifenphos (Hinosan).

Table -21

Antifungal activity of 6-chloro/bromo/nitro saligenin cyclic Phosphoramidothionates against H. oryzae



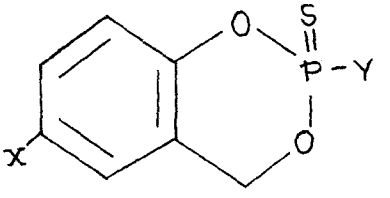
where, X = Cl, Br, NO_2

Y = alkylamido group, alkoxy group

Code No.	ED_{50} (μg/ml) (after 72 hours)	ED_{95} (μg/ml) (after 72 hours)
CL-6	3.69	21.68
CL-10	0.38	2.64
CL-24	4.351	49.10
BR-6	3.14	23.43
BR-10	2.27	23.99
BR-24	7.08	123.03
BR-27	14.14	1963.813
BD-25	257.23	546.35
BD-29	127.63	426.00
Edifenphos (Standard)	81.28	269.15

Table - 22

Antifungal activity of 6-chloro/bromo/nitro saligenin cyclic Phosphoramidothionates against *P. oryzae*



where, X = Cl, Br, NO_2
Y = alkylamido group, alkoxy group

Code No.	ED_{50} (μg/ml) (after 72 hours)	ED_{95} (μg/ml) (after 72 hours)
CL-6	0.84	4.71
CL-10	0.58	3.99
CL-24	2.85	24.33
BR-6	0.41	2.31
BR-10	1.21	7.70
BR-24	4.47	69.53
BR-27	4.07	85.69
BD-25	12.60	85.94
BD-29	12.65	78.24
Edifenphos (Standard)	< 5.00	$> 12.50, < 25.00$

9. Phytotoxic properties:

The Table - 23 shows the phytotoxic effect of the phosphoramidothionates on Triticum spp. (U.P. 262 variety).

The compound have no effect on the germination of wheat seed (Triticum spp.) upto 500 ppm.

Table - 23

Effect of chloro/bromo/nitro saligenin cyclic compounds on Germination of Wheat seed (Triticum spp.)

Code No.	Percent germination at different concentration		
	500 ppm	250 ppm	100 ppm
CL-6	100	100	100
CL-10	100	100	100
CL-24	100	100	100
BR-6	100	100	100
BR-10	100	100	100
BR-24	100	100	100
BR-27	100	100	100
BD-1	100	100	100
BD-5	100	100	100
BD-9	100	100	100
BD-25	100	100	100
BD-29	100	100	100
BD-8	100	100	100

10. Chemical Hydrolysis:

The chemical hydrolysis of BD-1, BD-5, BD-9 and BD-8 were carried out in phosphate buffer (pH 7.7) and of other compounds were performed in 9.5 mM NaOH in 50% ethanol (pH 11.85) at 30°C at λ =400, 410 and 420 nm for nitro compounds and λ = 294, 298 and 300 nm for chloro and bromo compounds. Three sets of experiments in each case have been performed. The hydrolysis data for the compounds at each wave length and the value of K_{hyd} calculated there of for a particular set are given in Appendix , Expt. No. 62-88 (pp 295 to 321) and $T_{\frac{1}{2}}$ are shown in Table - 24.

The average K_{hyd} increases in the order:

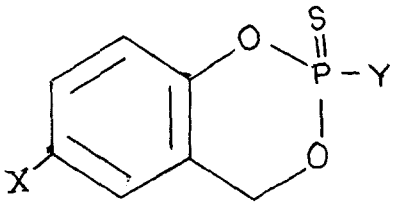
CL-6 < CL-10 < BR-10 < BD-25 < BD-29 < BR-6 < BD-5 < BD-1 < BR-27 < CL-24 < BR-24 < BD-9

i.e. the Phenoxy compound (BD-9) is least stable and the chloro compound with N,N-Diisobutylamido compound is most stable. Comparison of stability to alkaline hydrolysis of (CL-6, BR-6), (CL-10, BR-10) and (CL-24, BR-24) it is observed that the 6-chloro saligenin cyclic phosphoramidothionates are most stable and 6-bromo saligenin cyclic phosphoramidothionates are least stable to alkaline hydrolysis. The di-substituted compounds are more stable than the mono substituted compounds. Hence, it may be assumed that the rate of hydrolysis of the 6-substituted saligenin cyclic phosphoramidothionates are not only affected by the nature of the exocyclic substituents on the phosphorus atom but also it is significantly influenced by the nature of substituents present

at 6-position of the saligenin cyclic phosphoramidothionates.

Table - 24

Hydrolysis of 6-chloro/bromo/nitro saligenin cyclic phosphoramidothionates



where, X = Cl, Br, NO_2
Y = alkylamido group, alkoxy group

Code No.	X	Y	Average K_{hyd} $(min^{-1})x10^3$	$T_{\frac{1}{2}}$ (hours)
CL-6	Cl	N,N-Diisobutylamido	56.70	2037.03
CL-10	Cl	N,N-Dibutylamido	8850.0	1305.08
CL-24	Cl	N-Butylamido	6.218	1.857
BR-6	Br	N,N-Diisobutylamido	14.89	77.568
BR-10	Br	N,N-Dibutylamido	172.3	670.34
BR-24	Br	N-Butylamido	6.985	1.65
BR-27	Br	N-Hexylamido	5.834	1.979
BD-1	NO_2	Methoxy-ethoxy	4.125	2.80
BD-5	NO_2	Isopropoxy	1956.0	5.90
BD-9	NO_2	Phenoxy	9.692	1.191
BD-25	NO_2	N,N-Diisobutylamido	354.3	325.9
BD-29	NO_2	N,N-Dibutylamido	388.0	297.68
BD-8	NO_2	Methoxy	5.187	2.226

GENERAL CONCLUSION AND REMARKS

(i) Some 2-alkyl amido/alkoxy-6-chloro/bromo/nitro-4H-1, 3,2-benzodioxaphosphorin-2-sulphides have been prepared by the reaction of the corresponding phosphoramidodichloridothionates and phosphorothionates with 5-chloro/bromo/nitro saligenin at low temperature in presence of K_2CO_3 as dehydrogen chloride agent. Except CL-10, BR-10 and BR-27, all compounds were crystalline solids. The above mentioned three compounds were liquid in nature.

(ii) The structure of these compounds were elucidated by chemical analysis viz. UV, IR, Mass, ^{1}H NMR, ^{31}P NMR, ^{13}C NMR spectra.

All compounds showed characteristic IR bands for P-O-C (alkyl), P-O-C (aryl), P=S groups. Neither of the two P=S bands show any systematic shift which reflects changes in the inductive properties of the substituents, this is not unexpected if they do arise from mixed modes.

In the mass spectra, all compounds showed parent molecular ion (M^+) peaks. Fragmentation by the loss of 'SH' radical is important, most of the compounds show an ion due to $(M-{}^{\cdot}SH)^+$ and it is the base peak for the four compounds out of the twelve compounds.

(iii) The isopropoxy compound (BD-5) shows about 1.5 times, the methoxy-ethoxy about 3.5-5 times greater insecticidal activity compared to salithion, but in case chloro derivative of N-butyl amido compound (CL-24) the insecticidal activity is almost similar to that of salithion. The other compounds are almost non insecticidal. For Blow-fly and Grasshopper, the methoxy compound is more

active than salithion, the isopropoxy compound have some insecticidal activity, the methoxy-ethoxy compound also have some insecticidal activity in Grasshoppers. For Aphids the methoxy compound (BD-8) is the most active and phenoxy compound (BD-9) has least insecticidal activity.

(iv) All compounds are less toxic to rat compared to salithion. The isopropoxy compound (BD-5) and the methoxy compound (BD-8) have greater toxicity compared to other compounds. The CL-6, BR-10, BR-24 and BR-27 showed some apparent toxic symptoms but they recovered soon, or if died, it was after more than one month.

(v) The Histopathological study of some organs of compound-treated rat, showed the toxic symptoms.

The N,N-diisobutyl amido compound (CL-6) was only used for the tests. The section of kidney showed enlarged, midly dialated tubules with ruptured walls. The renal tubules were elongated, enlarged lumen, ruptured epithelial lining. The glomerulus were disorganised and Bowman's capsules were very reduced. The section of liver showed the vacucles, ruptured cell membranes, some cells without nuclei. The sections of intestine, lung and spleen showed no toxic symptoms.

(vi) Only four compounds (CL-6, BR-6, BD-5 and BD-8) have studied for acute oral toxicity and delayed neurotoxicity in hens. In these compounds BD-8 is the most toxic. Permanent paralysis in legs is observed only in case of N,N-diisobutyl amido compound (CL-6), upon histopathological examinations demyclination of sciatic nerve is found. In other compounds, permanent paralysis is not observed and the histopathological observation of sciatic nerves are normal.

(vii) Acetylcholinesterase inhibition data shows that the compound BD-1 (methoxy-ethoxy) has highest and CL-6 (N,N-diisobutyl amido) has lowest inhibitory activity for BFAChE. In case of goat whole blood, the anticholinesterase activity of the compound BD-25 (N,N-diisobutyl amido) is highest and BR-6 (N,N-diisobutyl amido) is lowest.

(viii) The antifungal activity study (by growth inhibition method) against H. oryzae and P. oryzae indicate that the chloro and bromo compounds show very good inhibitory effects on the growth of both the fungi H. oryzae and P. oryzae, their inhibitory effects are almost comparable to that of Ediphenphos (Hinosan).

(ix) The compounds are not phytotoxic to Triticum spp, upto the concentration 500 ppm.

(x) The rate of hydrolysis of the compounds are greatly influenced by the nature of the substituent at the 6-position of the benzodioxaphosphorin ring. It is observed that the 6-chloro saligenin cyclic phosphoramidothionates are most stable and 6-bromo saligenin cyclic phosphoramidothionates are least stable to alkaline hydrolysis.

(xi) The biological activities and other data justify further examination of methoxy and isopropoxy compound as potential insecticide and the chloro and bromo compounds as potential fungicide. Whether the use of these cyclic phosphorus compounds will protect the plants from pests and diseases in the field remains to be studied. In order to find out the chemical structure-biological activity relationship in these compounds we have to

synthesize several new compounds in which different group is to be incorporated in different positions of the aromatic ring and to investigate their biological activity. Besides, structural elucidation in regard to the conformation of the dioxaphosphorin ring from temperature dependent NMR and X-ray crystal structure would clarify the chemical structure-antifungal activity mechanism (and/or esterase inhibition mechanism) so that their selectivity of action can be known, thereby helping us to design selective and biodegradable potential pesticide.

REFERENCES

1. Vogel, I.A. : A Text Book of Practical Organic Chemistry, 3rd Edn., ELBS and Longman Ltd., London (1957).

2. Maeller, T., Birch, H.J. and Nielsen, N.C. : Inorganic Synthesis, Vol. IV, Ed. Bailar, J.C. Jr., McGraw-Hill Book Co., INC., New York, p. 71-73, (1953).

3. Buchler, C.A., Kirchner, F.K. and Leebel, G.F. : Organic Synthesis, Collective Vol. 3, Ed. Horning, E.C., John Wiley and Sons, INC, New York, p. 468, (1960).

4. Busvine, J.R. : A critical review of the techniques for testing insecticides, Pub. by Commonwealth Agricultural Bureaux, p. 77, (1971).

5. McIntosh, A.H. : J. Sci. Food Agri., 12, 312, (1961).

6. Eto, M., Kishimoto, K., Matsumura, K., Oshita, N. and Oshima, Y. : Agri. Biol. Chem., 30, 181, (1966).

7. Eto, M., Chima, Y., Kitakato, S., Tanaka, F., and Kajima, K. : Botyu-Kagaku, 31, 33, (1965).

8. Busvine, J.R. : A Critical Review of the Techniques for Testing Insecticides, p. 75, (1971).

9. Woodward, G. : Principles in Drug Administration Methods of Animal Experimentation, Vol. 1, Ed. by Gay, W.I., Academic Press, New York, p. 351, (1965).

10. Upholt, W.M. : Federal Register, 37, 19383, (1972).

11. Hallingshaus, J.G., Abu-El-Haj, S., Fukuto, T.R. : J. Agric. Food Chem., 27(6), 1197, (1979).

12. Kramer, D.N. and Gamson, R.M. : Anal. Chem., 30, 251-254, (1958).

13. Porter, P.E., Sun, Y.P. and Archer, T.E. : Analytical methods for Pesticides, Plant Growth Regulators and Food Additive, Vol. II, Ed. by Zweig, G. Academic Press, New York, p. 351-374, (1964).

14. Nene, Y.L. and Thapliyal, P.N. : Fungicides in Plant Disease Control, Oxford and IBM, New Delhi, India, p. 413, (1979).

15. Vincent, J.M. : Nature, 159, p. 850, (1927).

16. Eto, M., Eto, T. and Oshima, Y. : Agrl. Biol. Chem., 26, p. 630, (1962).

17. Eto, M. : Organo phosphorus Pesticide; Organic and Biological Chemistry, CRC Press, Cleveland, Ohio, p. 74, (1974).

18. Das, B.K. : D.Sc. Thesis, Calcutta University, (1981).

19. Eto, M., Kobayashi, K., Keto, T. and Kojima, K. : Agri. Biol. Chem., 29(3), 243-248, (1965).

20. Thomas, L.C. : Interpretation of IR spectra of Organo-phosphorus Compounds, Heyden, London, (1974).

21. Bellamy, L.J. : The Infrared Spectra of Complex Molecules, Vol. I. 3rd Edition, Chapman and Hall, London, 347-364, (1975).

22. Colthup, N.B., Daley, L.H., Wiberly, S.E. : Introduction to Infrared and Raman Spectroscopy, Academic Press, 224, (1964).

23. Das, B.K. : N.B.U. Review (Sci. and Techno.), 2, 1, (1981).

24. Das, B.K., Das, D.K., Sarkar, A. and Shoolary, J.N. : N.B.U. Review (Sci. and Techno), 1, 2, (1980).

25. Oshima, Y. and Eto, M. : U.S. Patent 3, 478, 133, (1969).

26. Cooks, R.C. and Gerrad, A.F. : J. Chem. Soc. B, 1327, (1968).

27. Jörg, J., Houriet, R. and Spiteller, G. : Mh. Chem., 97, 1064, (1966).

28. Damico, J.N. : J. Assoc. Offic. ~~Anal.~~ Anal. Chem., 49, 1027, (1966).

29. Damico, J.N., Barron R.P. and Sphen, J.A. : Intern. J. Mass Spectrum Ion Phys., 2, 161, (1969).

30. Gills, R.G. and Occoclowitz, J.L. : Analytical Chemistry of Phosphorus Compounds, Ed. Halmann. M., Interscience, New York, (1972).

31. Djerassi, C., Budzikiewiez, H. and Williams, D.H. : Mass Spectra of Organic Compounds, San Francisco, Holden Day, (1967).

32. Eto, M., Kobayashi, K., Sasamoto, T., Cheng, H.M., Aikawa, T., Kume, T. and Oshima, Y. : Botyu-Kagaku, 33, 73-77, (1968).

33. Eto, M. : Organophosphorus Pesticides; Organic and Biological Chemistry, CRC Press, Cleveland, Ohio, 123, (1974).

34. Kadola, T., Okuno, Y., Miyamoto, J. : Botyu-Kagaku, 40, 49, (1975).

35. Busvine, J.R. : A critical review of the techniques for testing insecticides, Pub. by the Commonwealth Agricultural Bureaux, England, p. 267, (1971).

CHAPTER - 3

CYTOLOGICAL EFFECTS OF SOME PESTICIDES ON PLANTS AND ANIMALS
(a preliminary study)

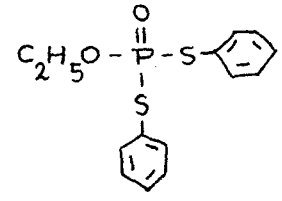
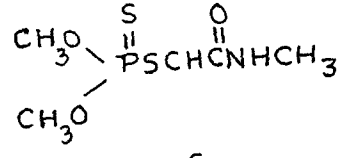
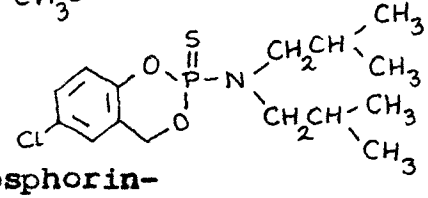
The biocides of the environment are known to induce cytological abnormalities in plant and animal cells. Especially the somatic cell abnormalities induced by fungicides and insecticides have been worked out by many workers[1-4].

The ability of some pesticides, to induce chromosomal abnormalities were also demonstrated in higher plant by many research workers for e.g. in Tradescantia sp. and Vicia faba by the use of mercurial fungicide Panogen-15[5] in Allium cepa by the use of two systemic fungicides Plantvax and Vitavax[6] and in Vicia faba by the use of insecticides Rotenone[7] and Dichlorvos[8].

The present study reports the mitotic effects on plant (Allium cepa) and animal (mice) with special emphasis on chromosomal abnormalities induced by the well known organophosphorus pesticides. Hinosan (Ediphenphos)(O-ethyl-S, S-diphenyldithiophosphate), Rogor (Dimethoate) [0,0-Dimethyl-S-(N-methylcarbamoyl methyl) phosphorothionate] and a new compound CL-6 (2-N,N-Diisobutyl amido-6-chloro-4H-1,3,2-benzodioxaphosphorin-2-sulphide) synthesized in our laboratory.

Table - 1

Name, structure and manufacturers of organophosphorus pesticides

Serial No.	Common name and other names	Chemical name	Structure	Manufacturer
1.	Hinosan (Ediphenphos)	O-ethyl-S,S-diphenyl-dithiophosphate		Bayer India Ltd., Express Towers, Nariman Point, Bombay-400021.
2.	Rogor (Dimethoate)	O,O-Dimethyl-S-(N-methylcarbamoyl methyl)phosphoro-thiolothioate		Rallis Pesticides, Rallis India Ltd., 21, Ravelin Street, Bombay-400001.
3.	Cl-6 (N,N,Diisobutyl amido compound)	2-N,N-Diisobutyl-amido-6-chloro-4H-1,3,2-benzodioxaphosphorin-2-sulphide.		Not commercial. Synthesized by us.

MATERIALS AND METHODS

(a) Cytotoxicity test in Plant:

Fast growing young roots of Allium cepa treated with 50 ppm, 100 ppm and 200 ppm conc. of the compound CL-6, Edifenphos (Hinosan) and Rogor (Dimethoate) for one hour and two hours treatment. Control was maintained in all cases. The control as well as the treated roots were excised and fixed in freshly prepared Farmer's fluid for 2 hours and 30 minutes (one glacial acetic acid : three ethyl alcohol) and squashes were prepared according to the method of Darlington and La Cour (1962) employing aceto-orchine stain.

Approximately 200 cells from at least three meristems per replicate were screened to determine the frequency of aberrant cells. The experiments were setted up in duplicate. The slides were screened thoroughly for chromosomal aberration like chromatid bridges and laggards, tripolar and tetrapolar divisional chromosome in cells, unequal distribution of chromosomes etc. at anaphase. Care was taken to exclude incomplete cells so as not to have any confusion regarding aneuploid cells.

(b) Cytotoxicity test in animal:

Bone-marrow tissues of albino mice (Swiss strain), age group three months each, were used in the present investigation. Each animal was administered the pesticides through oral route, the doses of the compounds were described in the Tables-5 to Table -7 and each tests were duplicated. Bone-marrow cells were

fixed after 24 hours exposures of CL-6 (N,N-Diisobutylamido) compound and the commercially popular pesticides Edifenphos and Rogor in order to test the cytogenetic injury. Cytological slides from bone-marrow tissue were prepared as per usual colchicine-Citrate-acetic acid ethanol-air drying-Giemsa schedule. Aberrations were scored from the slides. The prepared slides were screened properly and photographs of the aberrant cells were taken and analyzed.

RESULTS AND DISCUSSION

(a) Cytotoxicity in Plant:

The main effects of the compound CL-6, Ediphenphos and Rogor, however, were found mostly in anaphase stages of the root-meristems of the Allium cepa. The used concentrations and duration of treatment caused chromosomal abnormalities. The frequency of abnormality increased with the increase of concentration applied and for the period of treatment (Table-2 to Table -4).

Root treatment in 200 ppm concentrations for 2 hours of CL-6, Ediphenphos and Rogor pesticides showed 14.8%, 9.5% and 10.94% chromosomal abnormalities. These three pesticides showed chromosomal abnormalities even in 50 ppm concentration for one hour treatment.

It was found that in some cells the chromatids separated from each other but did not migrate to the poles were mostly restituted to form tetrapolar cells (Fig. c'). A number of other

Table - 2

Mitotic analysis of following treatments of the N,N-Diisobutyl amido compound (CL-6) in *Allium cepa*.

	Treatment (ppm)	Total dividing cells	Bridge of chromosome	Chromatid Gap	Chromatid break	Laggard chromosomes	Tetra polar division of chromosome	Tripolar division of chromosome	Diagonal division of chromosomes	Unequal distribution of chromosomes	Aberration Total	Aberration %
1 hour treatment	Control	200	-	-	-	-	-	-	-	-	-	-
	50	205	-	-	-	2	-	-	2	-	4	1.95
	100	200	2	1	-	-	1	-	3	-	7	3.5
	200	210	-	2	1	2	-	-	2	1	8	3.8
2 hours treatment	50	210	-	-	-	3	1	-	4	1	9	4.28
	100	200	3	2	2	3	1	1	4	3	19	9.5
	200	215	5	5	2	7	3	1	6	3	32	14.8

Table - 3

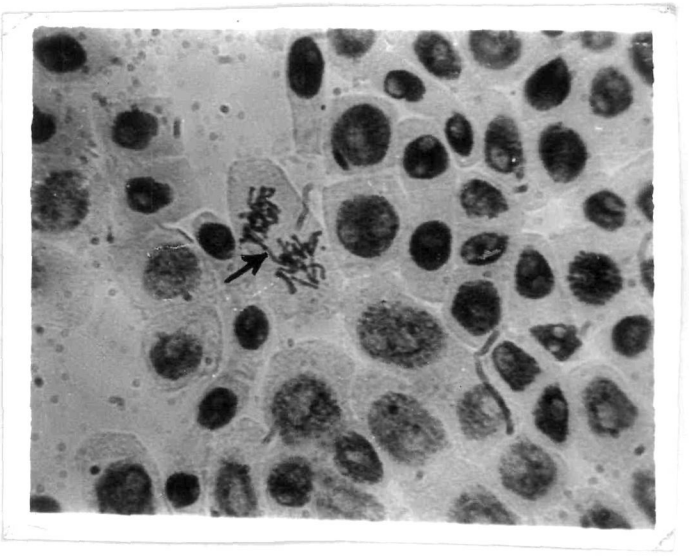
Mitotic analysis of following treatments of the Pesticide Ediphenphos in *Allium cepa*.

	Treatment (ppm)	Total dividing cells	Bridge of chromosome		Chromatid		Laggard chromosomes	Tetra-polar division	Tri-polar division	Dia-gonal division	Unequal distribution of chromosome	Aberration	
			Anaphase	Telophase	Gap	Break						Total	%
	Control	200	-	-	-	-	-	-	-	-	-	-	-
1 hour treatment	50	205	1	-	1	-	-	-	-	2	-	4	1.95
	100	203	2	-	1	1	1	-	-	2	-	7	3.44
	200	212	2	1	3	1	2	-	1	3	1	14	6.60
2 hour treatment	50	200	-	-	1	4	2	1	1	2	-	11	5.5
	100	211	2	1	2	3	3	1	1	5	1	19	9.00
	200	200	2	1	2	2	3	2	1	6	-	19	9.5

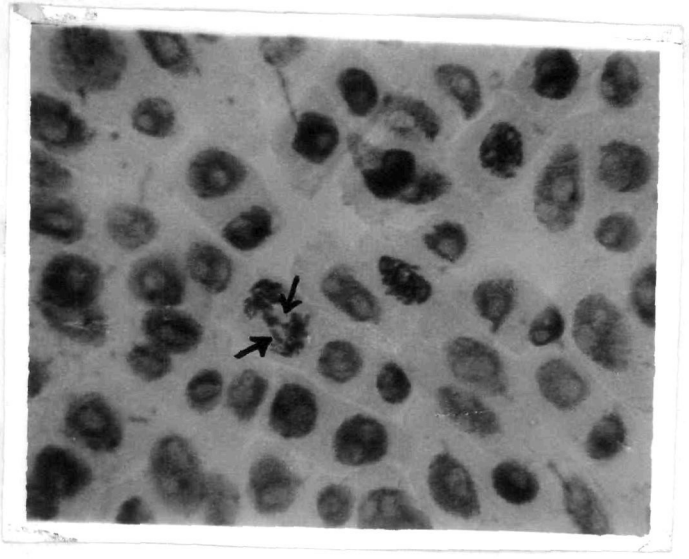
Table - 4

Mitotic analysis of following treatments of the Pestiside Rogor (Dimethoate) in *Allium cepa*

Treatment (ppm)		Total dividing cells	Bridge of chromosome		Chromatid		Laggard chromosomes	Tetra-polar division	Tri-polar division	Dia-gonal division	Unequal distribution of chromosome	Aberration	
			Anaphase	Telophase	Gap	Break						Total	%
	Control	200	-	-	-	-	-	-	-	-	-	-	-
1 hour treatment	50	200	-	-	1	1	1	-	-	3	-	6	3.0
	100	204	1	-	2	-	2	-	-	4	-	9	4.41
	200	220	2	1	2	2	1	-	-	3	-	11	5.0
2 hours treatment	50	220	3	1	2	2	2	-	1	5	-	16	7.27
	100	210	4	1	3	2	3	1	1	6	2	23	10.95
	200	201	4	2	2	1	4	1	2	5	1	22	10.94



(a')

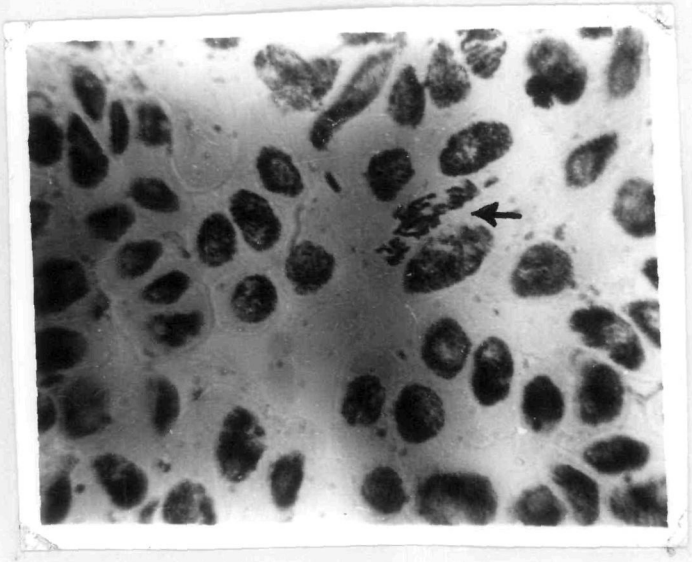


(b')

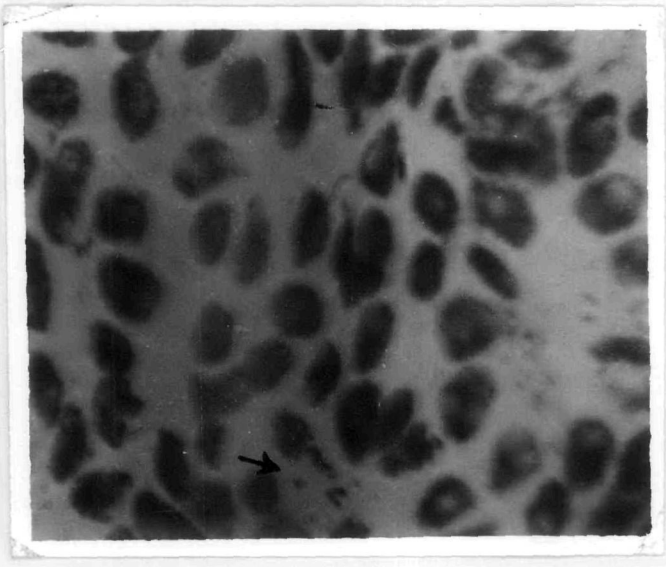
Figures showing different types of aberrant cells from root-tips of *Allium cepa* by treatment of Ediphenphos, Rogor and CL-6.

(a') Formation of bridge chromosome at anaphase stage.

(b') Formation of laggards and bridge chromosome at anaphase stage.



(c')

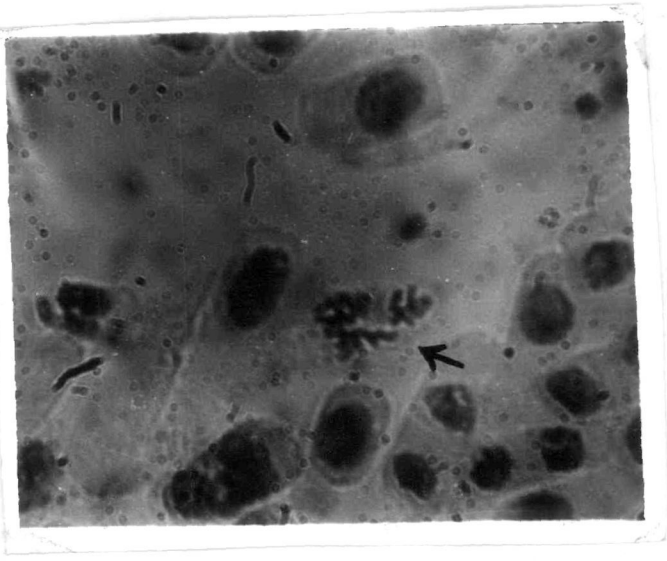


(d')

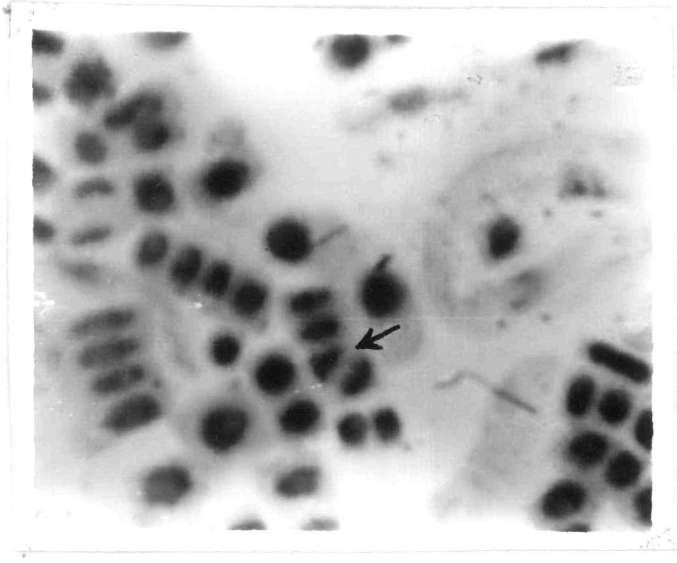
Figures showing different types of aberrant cells from root-tips of *Allium cepa* by treatment of Ediphenphos, Rogor and CL-6.

(c') The tetrapolar division of chromosomes at anaphase stage.

(d') The unequal distribution of chromosomes at two poles at anaphase stage.



(e')

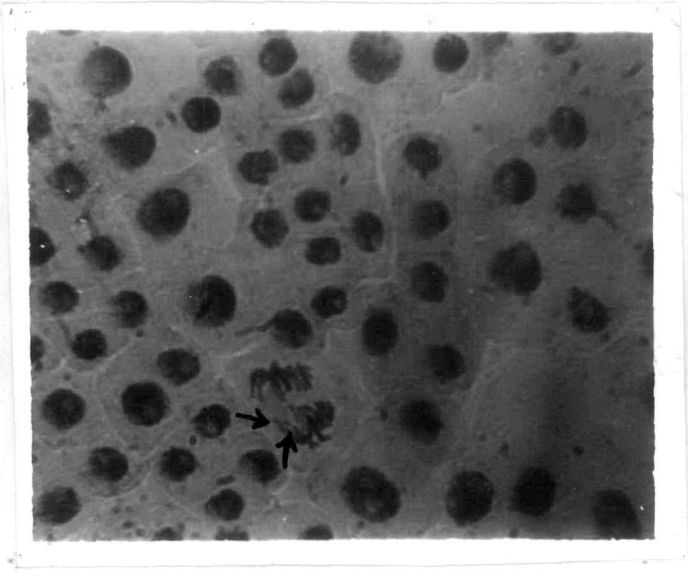


(f')

Figures showing different types of aberrant cells from root-tips of *Allium cepa* by treatment of Ediphenphos, Rogor and CL-6.

(e') The tetrapolar division at anaphase stage.

(f') The diagonal division at anaphase stage.



(g')

Figures showing different types of aberrant cells from root-tips of *Allium cepa* by treatment of Ediphenphos, Rogor and CL-6.

(g') Formation of gaps and breaks in the chromosomes at anaphase stage.

anaphase abnormalities were also noticed accompanied by normal anaphase. The other cases were that of disorientation of the spindle poles i.e. the two poles of the spindle fibres appeared to be located in two opposite corners instead of appearing parallel to the divided cells, thus forming what is known as diagonal anaphase organization (Fig. f'). The most prominant abnormality in anaphase stage was the formation of chromosome and chromatid bridges (Fig. a'). Laggard chromosomes were also noticed during anaphase (Fig. b'), disturbed anaphase gave rise to tripolar and tetrapolar cells (Fig. c' and Fig. e'). Sticky anaphase was also observed in very few number. All the above said aberrations, including the gap and breaks (Fig. g') in the chromosomes were found on application of CL-6, Ediphenphos and Rogor. The experimental data of the cytotoxic effects were described in the Table-2 to Table-4 and the photographs of the abnormal chromosomes were showed in the Fig.-a' to Fig.-g'.

(b) Cytotoxicity in animals (mice):

The present investigation provides information regarding the cytotoxic effects of Ediphenphos, Rogor and the newly synthesized compound CL-6 on the bone-marrow cells of mice in vivo and all the effects were observed in metaphase stages. In all the cases the administration period and route were same. Only the doses were changed, so we can say that the frequency of abnormality of chromosomes changed only for concentration of the compound. The Ediphenphos, Rogor and CL-6 showed the percentage

Table - 5

Effect of N,N-Diisobutylamido (CL-6) compound on bone marrow cells of mice:

Route	Dose (mg/kg)	Interval (in hour)	Total cell studied	Chromatid Gap	Chromatid Break	Formation of ring chromosome	Unequal chromatid stretching	Total aberrant cells	% of aberrant cells studied
Oral	50	24	300	2	1	3	-	6	2.00
Oral	30	24	300	1	1	2	-	4	1.33
Oral	10	24	300	-	-	1	-	1	0.33

Table - 6

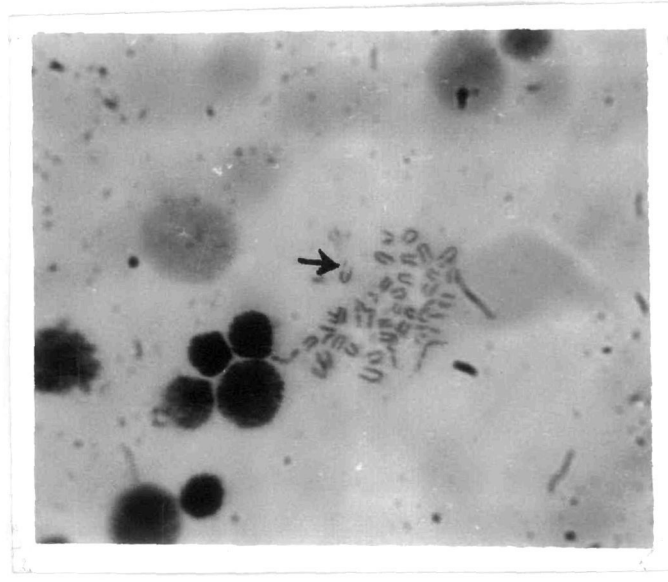
Effect of Ediphenphos on bone marrow cells of mice :

Route	Dose (mg/kg)	Interval (in hour)	Total cell studied	Chromatid		Formation of ring chromosome	Unequal chromatid stretching	Total aberrant cells	% of aberrant cells studied
				Gap	Break				
Oral	50	24	300	3	2	1	1	7	2.33
Oral	30	24	300	2	1	-	-	3	1.00
Oral	10	24	300	-	-	-	1	1	0.33

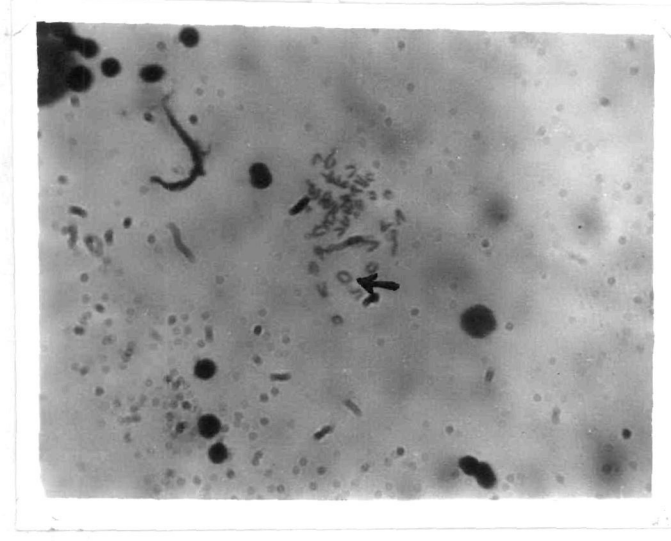
Table - 7

Effect of Rogor on bone marrow cells of mice :

Route	Dose (mg/kg)	Interval (in hour)	Total cell studied	Chromatid		Formation of ring chromosome	Unequal chromatid stretching	Total aberrant cells	% of aberrant cells studied
				Gap	Break				
Oral	50	24	300	2	1	2	-	5	1.66
Oral	30	24	300	1	3	1	-	5	1.66
Oral	10	24	300	1	-	1	-	2	0.66



(h')

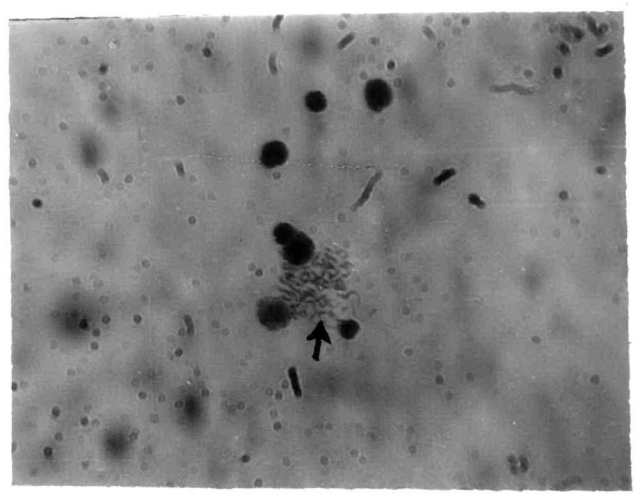


(i')

Figures showing different types of aberrant cells from bone-marrow cells of mice by treatment of Ediphenphos, Rogor and CL-6.

(h') Formation of gap in the chromosomes at metaphase stage.

(i') Formation of ring and break in the chromosome at metaphase stage.



(j')

Figures showing different types of aberrant cells from bone-marrow cells of mice by treatment of Ediphenphos, Rogor and CL-6.

(j') The unequal chromatic stretching at metaphase stage.

of aberrant cell studied after the application of dose 50 mg/kg were 2.33%, 1.66% and 2.00% and the dose 10 mg/kg were 0.33%, 0.66% and 0.33% respectively (Table -5 to Table - 7). It showed the physiological effect like uneven stretching of chromatid material (Fig. j'). In addition to physiological effect, it also showed gap and breaks (Fig. h'). The formation of rings (Fig. i') were very prominent. The mechanism of action of the compound CL-6 metabolites at nucleic acid level is not known. However, organophosphorus pesticides are known for their alkylating properties (Wild 1975). So DNA alkylation might be one of the reason for the production of chromosomal aberrations by the chemical. Effect like uneven stretching of chromatin material indicates that the pesticides may have acted upon the protein moiety of the chromosomes. Only chromatid type aberrations indicate that the chemical acted upon stages following G_1 stage of the cell cycle. For full confirmation of the above said aberrations, some sophisticated experiments and further study are needed.

Conclusion:

Cytotoxic effects of three pesticides (CL-6, Ediphenphos and Rogor) have been studied on the root-mitosis of *Allium cepa* and bone marrow chromosomes of mice *in vivo*. Dose, route and duration of exposure highly influence the aberration frequency. The chemicals have been found to be mutagenic in the present test system. However further studies are needed.

R E F E R E N C E S

1. Reddy, P.A. and Rao, B.V.R., Cytologia 34, 408, (1969).
2. Paramohan, S., Curr. Sci. 44, 613, (1975).
3. Kumar, C.V., Ramiah, M.J. and Subadra, J., Indian J. Exp. Biol. 15, 1071, (1977).
4. Sahu, R.K., Behera, B.N. and Sharma, G.B.S.R., The Nucleus 24, 60, (1981).
5. Ahmed, M. and Grant, W.F., Cytological effects of the mercurial fungicide-15 on Tradescantia sp. and Vicia faba root tips. Mutation Research, 14, 391-396, (1972).
6. Mohan, S.T., Cytological effects of fungicides Plantvax and Vitavax on somatic cells of Allium cepa, Curr. Sci., 44(22), 813-814, (1975).
7. Amer, S.M. and Michael, E., Cytological effects of pesticides XVI, Effect of the insecticide "Katenone" on root-mitosis of Vicia faba, Cytologia, 51, 171-176, (1986).
8. Amer, S.M. and Ali, S.M., Cytological effects of Pesticides XVII, Effect of the insecticide "Trichlorvos" on root-mitosis of Vicia faba, Cytologia, 51, 21-25, (1986).

APPENDIX

Experiment -1

Insecticidal activity of Cl-6 on Aphid (*Lipaphis erysimi*) by contact method

Conc. of the compound (µg/ml)	log. conc. of the compound (X)	% mortality 24h	% mortality 48 h
10	1.000	90	97
9	0.954	87	90
8	0.903	85	88
7	0.845	82	83
6	0.778	70	77
5	0.698	65	70
4	0.602	61	68
3	0.477	55	61
2	0.301	40	49
ED_{50} =		2.7697	2.1480
ED_{95} =		11.7230	10.5062
Regression constants : Y = mX + c			
m =		71.8165	65.2748
C =		18.2253	28.3253
r =		0.9895	0.9905

Experiment -2

Insecticidal activity of Cl-10 on Aphid (*Lipaphis erysimi*) by contact method

Conc. of the compound µg/ml	log. conc. of the compound (X)	% of mortality 24h	48h
10	1.000	95	98
9	0.954	84	88
8	0.903	79	82
7	0.845	75	79
6	0.778	70	75
5	0.698	65	71
4	0.602	51	59
3	0.477	43	47
2	0.301	34	38
	ED_{50} =	3.4479	3.0013
	ED_{95} =	11.6862	10.5118
Regression constants : Y = mX + C			
	m =	84.8886	82.6667
	C =	4.3667	10.5418
	r =	0.9871	0.9897

Experiment-3

Insecticidal activity of Cl-24 on Aphid (*Lipaphis erysimi*) by contact method

Conc. of the compound (µg/ml)	log. conc. of the compound (X)	% of mortality	
		24h	48h
10	1.000	92	95
9	0.954	80	90
8	0.903	76	81
7	0.845	62	71
6	0.778	55	64
5	0.698	50	60
4	0.602	40	57
3	0.477	33	45
2	0.301	30	32
ED_{50} =	4.3984	2.4372	
ED_{95}=	14.1742	15.0556	

Regression constants : Y = mX + C

m =	88.5596,	56.9025
C =	-6.9697,	27.9815
r =	0.9483,	0.8107

Experiment-4

Insecticidal activity of Br-6 against on Aphid (*Lipaphis erysimi*) by contact method.

Conc. of the compound µg/ml	Log. conc. of the compound (X)	% of mortality 24h	48h
10	1.000	80	86
9	0.954	72	84
8	0.903	70	80
7	0.845	68	75
6	0.778	66	70
5	0.698	58	66
4	0.602	44	59
3	0.477	38	50
2	0.301	25	31

ED_{50} =	4.2369	3.2237
ED_{95} =	16.0938	12.5677

Regression constants : Y = mX + C

m =	77.6396	76.1548
C =	1.3155	11.2863
r =	0.9893	0.9951

Experiment - 5

Insecticidal activity of Br-10 on Aphid (*Lipaphis erysimi*) by contact method

Conc. of the compound (µg/ml)	log. conc. of the compound (X)	% of mortality	
		24h	48h
10	1.000	80	90
9	0.954	72	80
8	0.903	60	75
7	0.845	55	71
6	0.778	50	65
5	0.698	46	59
4	0.602	40	55
3	0.477	37	52
2	0.301	30	35

ED_{50} =	4.9543	3.2931
ED_{95} =	23.8202	14.6277

Regression constants : Y = mX + C

m =	65.9867	69.4902
C =	4.1399	14.0315
r =	0.9376	0.9782

Experiment-6

Insecticidal activity of BR-24 on Aphid (*Lipaphis erysimi*) by contact method

Conc. of the compound (µg/ml)	log. conc. of the compound (X)	% of mortality 24h	48h
10	1.000	80	88
9	0.954	75	82
8	0.903	71	78
7	0.845	65	72
6	0.778	59	64
5	0.698	53	59
4	0.602	47	51
3	0.477	33	46
2	0.301	22	30
	ED_{50} =	4.5220	3.6512
	ED_{95} =	15.6796	13.2878
Regression constants : Y = mX + C			
	m =	83.3327	80.2118
	C =	-4.6106	4.8857
	r =	0.9978	0.9933

Experiment-7

Insecticidal activity of Bf-27 on Aphid (*Lipaphis erysimi*) by contact method

Conc. of the compound (µg/mL)	log. conc. of the compound (X)	% of mortality	
		24h	48h
10	1.000	77	85
9	0.954	72	83
8	0.903	70	80
7	0.845	65	70
6	0.778	50	65
5	0.698	45	64
4	0.602	44	58
3	0.477	35	40
2	0.301	29	31
	ED_{50} =	4.6930	3.5661
	ED_{95} =	19.8470	13.1649
Regression constants : Y = mX + C			
	m =	71.8573	79.3338
	C =	1.7511	6.1921
	r =	0.9637	0.9884

Experiment-8

Insecticidal activity of BD-1 on Aphild (*Lipaphis erysimi*) by contact method.

Conc. of the compound (µg/ml)	log. conc. of the compound (X)	% of mortality 24h	% of mortality 48h
10	1.000	89	92
9	0.954	82	86
8	0.903	76	82
7	0.845	72	75
6	0.778	56	65
5	0.698	53	60
4	0.602	39	54
3	0.477	36	45
2	0.301	30	33

ED_{50} =	4.2157	3.4739
ED_{95} =	13.5314	11.9284

Regression constants : $Y = mX + C$

m =	88.8497	83.9917
C =	-5.5196	4.5758
r =	0.9620	0.9900

Experiment -2

Insecticidal activity of BD-5 on Aphid (*Lipaphis erysimi*) by contact method

Conc. of the compound (µg/ml)	log. conc. of the compound (X)	% of mortality 24h	% of mortality 48h
10	1.000	92	95
9	0.954	78	80
8	0.903	65	76
7	0.845	61	71
6	0.778	54	66
5	0.698	50	60
4	0.602	41	56
3	0.477	38	41
2	0.301	32	34
	ED_{50} =	4.3779	3.5464
	ED_{95} =	16.6544	12.9229
Regression constants : Y = mX + C			
	m =	88.8497	83.9917
	C =	-5.5196	4.5758
	r =	0.9620	0.9900

Experiment - 10

Insecticidal activity of BD-8 on Aphid (*Lipaphis erysimi*) by contact method

Conc. of the compound (µg/mL)	log. conc. of the compound (X)	% of mortality	
		24h	48h
10	1.000	92	96
9	0.954	80	92
8	0.903	73	90
7	0.845	69	81
6	0.778	60	76
5	0.698	50	63
4	0.602	45	56
3	0.477	37	46
2	0.301	26	32
	ED_{50} =	4.2401	3.2843
	ED_{95} =	13.4162	9.7429
Regression constants : $Y = mX + C$			
	m =	89.9551	95.2902
	C =	-6.4361	0.7874
	r =	0.9773	0.9948

Experiment -II

Insecticidal activity of BD-9 on Aphid (Lipaphis erysimi) by contact method

Conc. of the compound µg/ml	log. conc. of the compound (X)	% of mortality	
		24h	48h
10	1.000	72	67
9	0.954	70	75
8	0.903	65	72
7	0.845	60	67
6	0.778	56	64
5	0.698	51	60
4	0.602	48	53
3	0.477	30	42
2	0.301	25	30
	ED_{50} =	4.8517	3.8358
	ED_{95} =	21.1982	20.7539
Regression constants : Y = mX + C			
	m =	70.2758	61.3621
	C =	1.7924	14.1764
	r =	0.9884	0.9677

Experiment - 12

Insecticidal activity of BD-25 on Aphid (*Lipaphis erysimi*) by contact method

Conc. of the compound µg/ml	log. conc. of the compound (X)	% of mortality 24h	% of mortality 48h
10	1.000	80	90
9	0.954	72	88
8	0.903	65	82
7	0.845	60	79
6	0.778	58	72
5	0.698	50	70
4	0.602	47	66
3	0.477	42	60
2	0.301	40	50
	ED_{50} =	3.9788	2.0790
	ED_{95} =	26.0310	13.2317
Regression constants : Y = mX + C			
	m =	55.1652	55.9869
	C =	16.9141	32.2042
	r =	0.9412	0.9900

Experiment -13

Insecticidal activity of BD-29 on Aphid (*Lipaphis erysimi*) by contact method

Conc. of the compound (µg/ml)	log. conc. of the compound (X)	% of mortality	
		24h	48h
10	1.000	90	92
9	0.954	80	90
8	0.903	76	88
7	0.845	70	80
6	0.778	60	72
5	0.698	55	58
4	0.602	45	50
3	0.477	40	46
2	0.301	35	42
	ED_{50} =	3.8636	3.1793
	ED_{95} =	14.2912	11.1675
Regression constant : Y = mX + C			
	m =	79.2156	82.4747
	C =	3.5005	8.5701
	r =	0.9661	0.9605

Experiment -14

Acetylcholinesterase Inhibition in Crysomaya megacephala (Blow fly) head homogenate (BFAChE) at C1-6 at 30°C

(Phosphate buffer pH = 8.0; total volume = 5.15 ml/1 fly head; λ = 625 nm; incubation time 30 mins.)

Sets	Inhibitor* Conc. (μg)	O.D.	Δ O.D.	% inhibition Y	I_{50} (M)
Control	-	0.660	-	-	
1	15	0.652	0.007	1.10	
2	30	0.636	0.024	3.63	
3	45	0.627	0.033	5.00	80.38×10^{-3}
4	60	0.613	0.047	7.12	
5	75	0.600	0.060	9.09	
6	90	0.587	0.073	11.06	

* X = log [Conc. of the inhibitor in μg]

Regression constants : Y = mX + C

m = 12.52

C = -14.58

r = 0.970

Experiment-15

Acetylcholinesterase inhibition in Chrysomeya megacephala (Blow fly) head homogenate (BFAChE) at 30°C of Cl_4-10

(Phosphate buffer pH = 8.0; total volume = 5.15 ml/1 fly head; λ = 625 nm; incubation time 30 mins.

Sets	Inhibitor* conc. (μg)	O.D.	Δ O.D	% Inhibition (Y)	I_{50} (M)
Control	0	0.691	-	-	
1	15	0.667	0.024	3.47	
2	30	0.646	0.045	6.51	
3	45	0.635	0.056	8.1	9.40×10^{-3}
4	60	0.611	0.080	11.57	
5	75	0.597	0.094	13.60	
6	90	0.580	0.111	16.06	

*X = Log [Conc. of the inhibitor in μg]

Regression Constants : Y = mX + C

C = -16.24

m = 15.81

r = 0.968

Experiment-16

Acetylcholinesterase Inhibition in Crysomaya megacephala (Blow fly) head homogenate (BFAChE) at Cl-24 at 30°C.

(Phosphate buffer pH = 8.0; total volume = 5.15 ml/1 fly head; λ = 625 nm; incubation time 30 mins.)

Sets	Inhibitor* Conc. (μg)	O.D.	Δ O.D	% inhibition Y	I_{50} (M)
Control	-	0.598	-	-	
1	15	0.575	0.023	3.846	
2	30	0.558	0.040	6.689	
3	45	0.532	0.066	11.037	3.1158×10^{-4}
4	60	0.513	0.085	14.214	
5	75	0.472	0.126	21.070	
6	90	0.453	0.145	24.247	
7	105	0.418	0.180	30.100	
8	120	0.385	0.213	35.619	

* X = log [Conc. of the inhibitor in μg]

Regression constants : Y = mX + C

m = 34.4649

C = -42.0219

r = 0.9309

Experiment - 17

Acetylcholinesterase Inhibition in Chrysomaya megacephala (Blow fly) head homogenate (EFAChE) at 30°C at BR-6

(Phosphate buffer, pH = 8.0; total volume = 5.15 ml/1 fly head, λ = 625 nm; incubation time 30 min.)

Sets	Inhibtor* Conc. (μg)	O.D.	△ O.D.	% Inhibition (Y)	I_{50} (M)
Control	-	0.416	-	-	
1	15	0.398	0.018	4.32	
2	30	0.384	0.032	7.69	
3	45	0.374	0.042	10.09	9.84×10^{-3}
4	60	0.370	0.046	11.05	
5	75	0.357	0.056	14.18	
6	90	0.347	0.069	16.58	

* X = log [Conc. of the inhibitor in μg]

Regression constants : Y = mX + C

C = -13.93

m = 14.88

r = 0.97

Experiment - 18

Acetylcholinesterase Inhibition in Chrysomaya megacephala (Blow fly) head homogenate (BFAChE) at 30°C of BR-10

(Phosphate buffer, pH = 8.0; total volume = 5.15 ml/1 fly head, λ = 625 nm; incubation time 30 min.)

Sets	Inhibitor* conc. (μg)	O.D	ΔO.D	% Inhibition (Y)	I_{50}(M)
Control	-	0.398	-	-	
1	15	0.376	0.022	5.52	
2	30	0.368	0.030	7.53	
3	45	0.358	0.040	10.05	3.15×10^{-3}
4	60	0.344	0.054	13.56	
5	75	0.332	0.066	16.58	
6	90	0.321	0.077	19.34	

* X = log [Conc. of the inhibitor in μg]

Regression constants : Y = mX + C

C = -17.03

m = 17.63

r = 0.95

Experiment -19

Acetylcholinesterase Inhibition in Crysomaya megacephala (Blow fly) head homogenate (BFAChE) of BS-24 at 30°C

(Phosphate buffer pH = 8.0; total volume = 5.15 ml/1 fly head, λ = 625 nm; incubation time 30 mins.)

Sets	Inhibition* conc. (μg)	O.D.	ΔO.D	% inhibition (Y)	I_{50} (M)
Control	-	0.598	-	-	
1	15	0.502	0.096	16.053	
2	30	0.465	0.133	22.241	
3	45	0.418	0.180	30.100	4.8976×10^{-5}
4	60	0.367	0.231	38.629	
5	75	0.321	0.277	46.321	
6	90	0.261	0.337	56.354	
7	105	0.225	0.373	62.374	
8	120	0.196	0.402	67.224	

* X = log [Conc. of the inhibitor in μg]

Regression constants : Y = mX + C

m = 59.1444

C -61.1957

r = 0.9577

Experiment-20

Acetylcholinesterase Inhibition in Crysomaya megacephala (Blow fly) head homogenate (BFAChE) at B.P-27 at 30°C

(Phosphate buffer, pH = 8.0; total volume = 5.15 ml/1 fly head; λ = 625 nm; incubation time 30 mins.)

Sets	Inhibitor* Conc. (μg)	O.D.	ΔO.D	% inhibition Y	I_{50} (M)
Control	-	0.355	-	-	
1	15	0.331	0.024	6.760	
2	30	0.314	0.041	11.549	
3	45	0.290	0.065	18.310	
4	60	0.212	0.143	40.282	4.8277×10^{-5}
5	75	0.195	0.160	45.070	
6	90	0.174	0.181	50.986	
7	105	0.155	0.200	56.338	
8	120	0.136	0.219	61.690	

*X = log [Conc. of the inhibitor in μg]

Regression constants : Y = mX + C

m = 66.5126

C = -80.1419

r = 0.9558

Experiment-21

Acetylcholinesterase Inhibition in Chrysomaya megacephala (Blow fly) head homogenate (BFAChE) at 30°C of BD-1

(Phosphate buffer, pH = 8.0; total volume = 5.15 ml/1 fly head, λ = 625 nm; incubation time 30 mins.)

Sets	Inhibitor * Conc. (μg)	O.D.	Δ O.D.	% Inhibition (Y)	I_{50}(M)
Control	-	0.580	-	-	
1	1.128	0.275	0.305	52.5	
2	0.564	0.320	0.260	44.8	
3	0.423	0.345	0.235	40.5	5.112×10^{-4}
4	0.232	0.390	0.190	32.7	
5	0.141	0.455	0.125	21.6	
6	0.0564	0.510	0.070	12.0	

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

Regression constants = Y = mX + C

m = 0.0286

C = -1.5276

r = 0.995

Experiment No.-22

Acetylcholinesterase inhibition in Blow fly (Chrysomaya megacephala) head homogenate (BFAChE) of BD-5 at 30°C

(Phosphate buffer, pH 8.0; total volume = 5.15 ml/1 fly head, λ = 625 nm; incubation time 30 min)

Sets	Inhibitor* Conc. (μg)	O.D.	Δ O.D	% Inhibition (Y)	I_{50} (M)
Control	-	0.850	-	-	
1	0.605	0.790	0.060	7.0	
2	1.21	0.670	0.180	21.0	
3	2.42	0.510	0.340	40.0	0.25×10^{-5}
4	3.63	0.400	0.450	52.9	
5	6.05	0.300	0.500	64.7	
6	9.075	0.260	0.590	69.0	
7	12.1	0.250	0.600	70.5	

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

Regression constants $Y = mX + C$

$C = -22.2767$

$m = 32.8057$

$r = 0.9625$

Experiment-23

Acetylcholinesterase inhibition in Chrysomaya megacephala (Blow fly) head homogenate (BFAChE) of BD-9 at 30°C

(Phosphate buffer, pH 8.0; total volume = 5.15 ml/1 fly head, = 625 nm; incubation time 30 min.)

Sets	Inhibition conc. (μg)	O.D	$\triangle$ O.D.	% Inhibition (Y)	I_{50} (M)
Control	-	0.420	-	-	
1	2.529	0.200	0.220	52.3	
2	1.264	0.245	0.176	41.7	
3	0.843	0.265	0.155	37.0	1.337×10^{-6}
4	0.632	0.290	0.130	30.9	
5	0.421	0.315	0.105	25.0	
6	0.210	0.345	0.075	18.0	
7	0.0843	0.380	0.040	9.5	

* X = log [Conc. of the inhibitor in μg]

Regression constants : Y = mX + C

m = 0.0304

C = -1.1746

r = 0.996

Experiment -24

Acetylcholinesterase inhibition in Chrysomaya megacephala (Blow-fly) head homogenate (BFAChE) of BD-25 at 30°C.

(Phosphate buffer, pH = 8.0; total volume = 5.15 /1 fly head; λ = 625 nm; incubation time 30 min).

Sets	Inhibitor* Conc. (μg)	O.D.	ΔO.D	% inhibition (Y)	I_{50}(M)
Control	-	1.016	-	-	
1	3.58	0.968	0.048	4.72	
2	7.16	0.904	0.112	11.02	
3	10.74	0.854	0.162	15.94	
4	14.32	0.798	0.218	21.55	2.76×10^{-5}
5	17.90	0.749	0.267	26.28	
6	21.48	0.686	0.330	32.48	
7	25.06	0.637	0.379	37.30	
8	28.64	0.576	0.440	43.31	
9	32.22	0.523	0.493	48.52	

*X = log [Conc. of the inhibitor in μg]

Regression constants : Y = mX + C

m = 44.249

C = 251.72

r = 0.967

Experiment-25

Acetylcholinesterase Inhibition in Chrysomaya megacephala (Blow fly) head homogenate (BFAChE) at ED-29 at 30°C

(Phosphate buffer, pH 8.0; total volume = 5.15 m/1 fly head; λ= 625 nm, incubation time 30 mins.)

Sets	Inhibitor Conc. x 10^6(M)	Log inhibitor Conc. (X)	O.D.	△O.D	% inhibtion	I_{50}(M)
Control	-	-	1.001	-	-	
1	1.9417	-5.7118	0.664	0.337	33.67	
2	3.8835	-5.4108	0.627	0.374	37.36	
3	5.8285	-5.2345	0.589	0.412	41.16	
4	7.7669	-5.1097	0.554	0.447	44.66	9.48×10^{-6}
5	9.7087	-5.0128	0.514	0.487	48.66	
6	11.6504	-4.9336	0.475	0.526	52.55	
7	13.5922	-4.8667	0.442	0.559	55.84	
8	15.5340	-4.8087	0.400	0.601	60.04	

Regression Constants : $Y = mX + C$

$C = 194.67$

$m = 28.803$

$r = 0.9571$

Experiment-26

Acetylcholinesterase inhibition in Goat whole blood of Cl-6 at 30°C
(Phosphate buffer, pH = 8.0; total volume = 5.15 ml/0.2 ml blood splasma;
λ = 625 nm; incubation time 30 mins.)

Sets	Inhibitor* Conc. (μg)	O.D.	Δ O.D.	% inhibition (Y)	I_{50} (M)
Control	-	1.015	-	-	
1	15	1.010	0.005	2.75	
2	30	0.954	0.061	5.02	
3	45	0.921	0.094	7.58	4.25×10^{-3}
4	60	0.899	0.116	10.04	
5	75	0.881	0.134	12.51	
6	90	0.866	0.149	14.08	
7	120	0.843	0.172	17.53	

* X = log [Conc. of the inhibitor in μg]

Regression constants Y = mX + C

m = 18.32

C = -21.12

r = 0.967

Experiment -27

Acetylcholinesterase inhibition in goat whole blood of Cl-10 at 30°C
(Phosphate buffer, pH = 8.0; total volume = 5.15 ml/0.2 ml blood; λ = 625 nm, incubation time 30 mins.)

Sets	Inhibitor* Conc. (μg)	O.D.	Δ O.D.	% Inhibition (Y)	I_{50} (M)
Control	0	1.160	-	-	
1	15	1.120	0.04	3.50	
2	30	1.102	0.058	5.00	
3	45	1.077	0.083	7.10	
4	60	1.053	0.107	9.20	4.13×10^{-3}
5	75	1.030	0.130	11.20	
6	90	0.992	0.167	14.43	
7	120	0.938	0.222	19.13	
8	150	0.914	0.246	21.20	

*X = Log [Conc. of the inhibitor in μg]

Regression constants : Y = mX + C

C = -21.28

m = 18.42

r = 0.938

Experiment - 28

Acetylcholinesterase inhibition in goat whole blood of CI-24 at 30°C
(Phosphate buffer, pH = 8.0; total volume = 5.15 ml/0.2 ml blood plasma;
λ = 625 nm; incubation time 30 mins.)

Sets	Inhibitor* Conc. (μg)	O.D.	Δ O.D	% inhibitor (Y)	I_{50} (M)
Control	-	0.453	-	-	
1	15	0.445	0.008	1.766	
2	30	0.422	0.031	6.843	
3	45	0.415	0.038	8.388	5.9967×10^{-3}
4	60	0.404	0.049	10.817	
5	75	0.399	0.054	11.920	
6	90	0.387	0.066	14.569	
7	105	0.379	0.074	16.335	
8	120	0.374	0.079	17.439	
9	150	0.364	0.089	19.647	

*X = log [Conc. of the inhibitor in μg]

Regression constants Y = mX + C
m = 17.6441
C = -19.7711
r = 0.9883

Experiment-29

Acetylcholinesterase Inhibition in goat whole blood of BR-6 at 30°C
(Phosphate buffer, pH = 8.0; total volume = 5.15 ml/0.2 ml blood plasma;
λ = 625 nm; incubation time 30 min.)

Sets	Inhibitor* μg.	O.D.	△ O.D.	% Inhibition (Y)	I_{50} (M)
Control	-	1.015	-	-	
1	15	1.004	0.011	1.08	
2	30	0.979	0.036	3.54	
3	60	0.949	0.066	6.50	28.93×10^{-3}
4	90	0.913	0.102	10.04	
5	120	0.883	0.132	13.00	
6	150	0.861	0.154	15.17	

* X = log [Conc. of the inhibitor in μg]

Regression constants : Y = mX + C
C = -16.54
m = 13.96
r = 0.975

Experiment-30

Acetylcholinesterase Inhibition in goat whole blood at 30°C of BP-10
(Phosphate buffer, pH = 8.0; total volume = 5.15 ml/0.2 ml blood plasma; λ = 625 nm; incubation time 30 min.)

Sets	Inhibitor* conc. (μg)	O.D.	△ O.D	% Inhibition (Y)	I_{50} (M)
Control	-	1.072	-	-	
1	15	1.023	0.049	4.57	
2	30	0.991	0.081	7.55	
3	60	0.938	0.134	12.50	1.22×10^{-3}
4	90	0.905	0.167	15.57	
5	120	0.830	0.242	22.57	
6	150	0.778	0.294	27.42	

* X = log [Conc. of the inhibitor in μg]

Regression constant : Y = mX + C

C = -23.31

m = 21.62

r = 0.946

Experiment-31

Acetylcholinesterase inhibition in goat whole blood (AChE) of BR-24 at 30°C
(Phosphate buffer, pH = 8.0; total volume = 5.15/0.2 ml blood plasma;
λ = 625 nm; incubation time 30 min.)

Sets	Inhibitor* conc. (μg)	O.D	Δ O.D	% inhibitor (Y)	I_{50} (M)
Control	-	0.418	-	-	
1	15	0.398	0.020	4.785	
2	30	0.381	0.037	8.852	
3	45	0.373	0.045	10.765	4.5548×10^{-3}
4	60	0.361	0.057	13.636	
5	75	0.354	0.064	15.531	
6	90	0.348	0.070	16.746	
7	105	0.342	0.076	18.182	
8	120	0.332	0.080	19.139	
9	150	0.326	0.092	22.009	
10					

* X = log [Conc. of the inhibitor in μg]

Regression constants : Y = mX + C

m = 16.9791

C = -16.1390

r = 0.9916

Experiment-32

Acetylcholinesterase inhibition in goat whole blood of BR-27 at 30°
(Phosphate buffer, pH = 8.0; total volume = 5.15 ml/0.2 ml blood plasma,
λ = 625 nm; incubation time 30 mins.)

Sets	Inhibitor* Conc. (μg)	O.D.	$\triangle$ O.D	% inhibition (Y)	I_{50} (M)
1	Control	0.276	-	-	
2	15	0.268	0.008	2.898	
3	30	0.262	0.014	5.072	
4	45	0.250	0.026	9.420	
5	60	0.243	0.033	11.956	4.4789×10^{-3}
6	75	0.236	0.040	14.493	
7	90	0.233	0.043	15.580	
8	105	0.231	0.045	16.304	
9	120	0.228	0.048	17.391	
10	150	0.222	0.054	19.565	

* X = log [Conc. of the inhibitor in μg]

Regression constants : Y = mX + C

m = 17.6356

c = -19.2035

r = 0.9883

Experiment - 33

Acetylcholinesterase inhibition in goat whole blood of BD-1 at 30°C
(Phosphate buffer; pH = 8.0; total volume = 5.15 ml/0.2 ml blood plasma;
λ = 625 nm; incubation time 30 min.)

Sets	Inhibitor* Conc. (μg)	O.D.	Δ O.D	% Inhibition (Y)	I_{50} (M)
Control	-	1.015	-	-	
1	15	0.999	0.016	1.57	
2	30	0.989	0.026	2.56	
3	60	0.974	0.041	4.03	4.09×10^{-3}
4	90	0.941	0.074	7.29	
5	120	0.933	0.082	8.07	
6	150	0.929	0.086	8.47	

* X = log [Conc. of the inhibitor in μg]

Regression constants : Y = mX + C
C = -18.01
m = 17.52
r = 0.995

Experiment - 34

Acetylcholinesterase inhibition in Goat whole blood (AChE) of BD-5 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	-	0.998	-	-	
1	15	0.803	0.195	19.54	
2	30	0.755	0.243	24.35	
3	45	0.708	0.290	29.06	10.72×10^{-5}
4	60	0.663	0.335	33.57	
5	75	0.617	0.381	38.18	
6	90	0.568	0.430	43.09	
7	105	0.521	0.477	47.80	

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

Regression constants $Y = mX + C$

C=-22.2767

m = 32.8057

r = 0.9625

Experiment - 35

Acetylcholinesterase inhibition in goat whole blood of BD-9 at 30°C

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

Sets	Inhibitor* Conc. (μg)	O.D.	Δ O.D	% inhibition (Y)	I_{50} (M)
Control	-	1.072	-	-	
1	15	0.979	0.093	8.67	
2	30	0.959	0.113	10.54	
3	60	0.938	0.134	12.50	1.15×10^{-3}
4	90	0.873	0.199	18.56	
5	120	0.830	0.242	22.57	
6	150	0.739	0.333	31.06	

* X = log [Conc. of the inhibitor in μg]

Regression constants : Y = mX + C

m = 19.80

C = -17.80

r = 0.89

Experiment -36

Acetylcholinesterase inhibition in goat whole blood of ED-25 at 30°C

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

Sets	Inhibitor* Conc. (μg)	O.D	Δ O.D	% inhibition (Y)	I_{50} (M)
Control	-	1.016	-	-	
1	0.668	0.971	0.045	4.43	
2	1.336	0.926	0.090	8.86	
3	2.004	0.885	0.131	12.89	1.409×10^{-5}
4	2.672	0.840	0.176	17.32	
5	3.840	0.793	0.223	21.95	
6	4.008	0.748	0.268	26.38	
7	4.678	0.697	0.319	31.40	

*X = log [Conc. of the inhibitor in μg]

Regression constants : Y = mX + C

m = 31.034

C = 178.45

r = 0.9567

Experiment -37

Acetylcholinesterase Inhibition in Goat whole blood of BD-29 at 30°C

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

Sets	Inhibitor for conc. $\times 10^{6}$ (M)	Log inhibitor Conc. (X)	O.D.	ΔO.D	% inhibition (Y)	I_{50} (M)
Control	-	-	1.221	-	-	
1	1.941	-5.711	0.881	0.340	27.27	
2	3.883	-5.410	0.358	0.363	29.73	
3	5.828	-5.234	0.832	0.389	31.86	
4	7.766	-5.109	0.801	0.420	34.40	
5	9.708	-5.012	0.778	0.443	36.28	4.16×10^{-5}
6	11.650	-4.933	0.753	0.468	38.33	
7	13.592	-4.866	0.723	0.498	40.79	
8	15.534	-4.808	0.689	0.532	43.57	
9	17.47	-4.757	0.657	0.564	46.19	

Regression constants : $Y = mX + C$

$C = 133.49$

$m = 19.061$

$r = 0.9569$

Experiment - 38

Effect of Cl-6 on growth of *H. oryzae*

Concentration (μg/ml)	Percentage of inhibition over control after			
	24 hours	48 hours	72 hours	96 hours
20	100	98.0*	92.5*	84.7*
10	87.2*	80.2*	75.2*	70.0*
5	72.2*	64.2*	58.0*	52.2*
2.5	57.5*	48.2*	39.7*	33.4*
1.25	43.2*	31.0*	22.5*	15.5*
0.625	28.2*	16.5*	13.2*	6.2
ED_{95} (μg/ml)	14.74	18.96	21.68	28.18
ED_{50} (μg/ml)	1.74	2.72	3.69	4.79
Regression constants : Y = mX + C				
m =	48.530	53.380	58.580	58.460
C =	38.290	26.780	16.730	10.230
r =	0.999	0.999	0.999	0.999

* Data used for regression analysis

Experiment - 39

Antifungal activity of Cl-10 against *H. oryzae*
(growth inhibition method)

Concentration (μg/ml)	Percentage inhibition over control after			
	48 hrs.	72 hrs.	96 hrs.	120 hrs.
12.0	100	100	100	100
6.0	100	100	100	96.50*
3.0	100	96.40*	86.60*	80.44*
1.50	94.50*	83.89*	79.33*	63.75*
0.75	72.82*	66.78*	60.80*	47.12*
0.375	55.23*	48.64*	42.28*	30.49*
ED_{95} (μg/ml)	1.56	2.64	3.78	5.57
ED_{50} (μg/ml)	0.32	0.38	0.48	0.85
Regression constants : $Y = mX + C$				
m =	65.23	53.28	50.33	54.93
C=	82.34	75.57	65.97	53.99
r =	0.969	0.991	0.966	0.990

*Data used for regression analysis.

Experiment-40

Effect of Cl-24 on growth of *H. oryzae*

Concentration (µg/ml)	Percentage of inhibition over control after			
	24 hours	48 hours	72 hours	96 hours
40	100	100	100	100
20	100	93.18*	77.27*	64.58*
10	63.63*	77.27*	65.83*	56.67*
5	54.54*	68.33*	56.25*	49.25*
2.5	50.00*	40.00*	35.42*	31.34*
1.25	45.45*	33.33*	28.12*	22.39*
ED_{95} (µg/ml)	465.4968	21.0724	49.1050	118.8796
ED_{50} (µg/ml)	2.3711	2.8889	4.3517	6.9243
Regression constants : Y = mX + C				
m =	19.6252	52.1448	42.7569	36.4452
C =	42,6416	25.9749	22.6927	19.3723
r =	0.9827	0.9829	0.9897	0.9871

*Data used for regression analysis.

Experiment - 41

Antifungal activity of BR-6 against *H. oryzae*
(growth inhibition method)

Conc. of the compound (μg/ml)	Percentage of inhibition over control after			
	24 hrs.	48 hrs.	72 hrs.	96 hrs.
20	100.00	100.00	96.20*	91.35*
10	88.00*	82.25*	77.00*	72.50*
5	74.00*	68.45*	61.30*	54.00*
2.5	58.50*	47.00*	42.00*	36.30*
1.25	46.50*	35.00*	28.00*	18.75*
0.625	32.25*	24.00*	16.00*	9.35
ED_{95} = (μg/ml)	15.00	18.82	23.43	24.16
ED_{50} = (μg/ml)	1.52	2.35	3.14	4.24
Regression constants : Y = mX + C				
m =	45.34	49.81	51.59	59.54
C =	41.65	31.51	24.33	12.65
r =	0.998	0.992	0.996	0.999

* Data used for regression analysis.

Experiment - 42

Antifungal activity of Br-10 against *H. oryzae* (growth inhibition method)

Conc. of the compound (μg/ml)	Percentage of inhibition over control after			
	24 hrs	48 hrs	72 hrs	96 hrs
20	100.00	100.00	94.00*	86.20*
10	90.50*	82.75*	77.50*	69.00*
5	79.00*	70.00*	65.50*	57.00*
2.5	69.50*	58.00*	52.00*	46.00*
1.25	57.75*	46.50*	40.00*	35.40*
0.625	38.00*	30.25*	24.00*	14.20*
ED_{95} = (μg / ml)	10.13	18.90	23.99	51.37
ED_{50} = (μg / ml)	1.00	1.66	2.27	3.15
Regression constants : Y = mX + C				
m =	44.76	42.68	43.99	37.13
C =	49.98	40.51	34.29	37.48
r =	0.995	0.998	0.993	0.999

* Data used for regression analysis.

Experiment-43

Effect of BR-24 on growth of *H. oryzae* (growth inhibition method)

Concentration (μg/ml)	Percentage of inhibition over control after			
	24 hours	48 hours	72 hours	96 hours
50	100	100	100	100
25	90.83*	88.33*	73.33*	63.71*
12.5	71.67*	61.66*	54.68*	41.79*
5	63.63*	56.67*	43.23*	34.32*
2.5	54.54*	46.67*	35.42*	26.12*
1.25	36.36*	32.29*	22.92	19.40*
ED_{95}(g/ml)	37.2608	53.6609	123.0332	376.4833
ED_{50}(g/ml)	2.4212	3.5575	7.0833	14.0610
Regression constants : Y = mX + C				
m =	37.9037	38.1839	36.2966	31.5186
C =	35.4437	28.9549	19.1393	13.8161
r =	0.9817	0.9622	0.9880	0.9624

* Data used for regression analysis.

Experiment - 44

Effect of BR-27 on growth of *H. oryzae*

Concentration (μg/ml)	Percentage of inhibition over control after			
	24 hours	48 hours	72 hours	96 hours
50	94.17 *	83.33 *	63.63 *	43.43 *
25	75.83 *	70.45 *	54.54 *	38.33 *
12.5	63.63 *	54.54 *	45.45 *	33.33 *
5	56.28 *	47.92 *	40.91 *	31.67 *
2.5	45.45 *	40.00 *	36.36 *	26.67 *
1.25	35.42 *	33.33 *	27.27 *	23.33 *
ED_{95} (μg/ml) =	73.8835	164.6996	1963.8131	1.2662×10^6
ED_{50} (μg/ml) =	3.5734	5.4418	14.1446	217.5413
Regression constants : Y = mX + C				
m =	34.2085	30.3859	21.0034	11.9523
C =	31.0798	27.6438	25.8337	22.0610
r =	0.9860	0.9812	0.9870	0.9883

* Data used for regression analysis.

Experiment - 45

Antifungal activity of BD-1 against *H. oryzae*
(growth inhibition method)

Conc. of the compound (μg/ml)	Percentage of inhibition over control after	
	48 hrs.	72 hrs.
2000	100	100
1600	100	81*
1200	76*	62*
1000	62*	49*
800	47*	33*
600	21*	12*
ED_{95} = (μg/ml)	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.

Experiment - 46

Antifungal activity of BD-5 against *H. oryzae*
(growth inhibition method)

Conc. of the compound (μg/ml)	Percentage of inhibition over control after	
	48 hrs	72 hrs
2000	65*	52*
1600	50*	40*
1200	33*	21*
1000	21*	11*
800	0	0
600	0	0
ED_{95} = (μg/ml)	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.

Experiment - 47

Antifungal activity of BD-9 against *H. oryzae* (growth inhibition method)

Conce of the compound (μg/ml)	% of inhibition over control after	
	48h	72h
2000	100	100
1600	100	100
1200	100	100
1000	97*	86*
800	88*	69*
600	72*	49*
400	57*	23*
ED_{95} = (μg/ml)	933.25	1148.15
ED_{50} = (μg/ml)	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.

Experiment - 48

Effect of BD-25 on the growth of *H. oryzae*

Concentration (μg/ml)	Percentage of inhibition over control after			
	24 hours	48 hours	72 hours	96 hours
500	100	100	91.5*	84.6*
400	100	94.5*	79.4*	71.2*
300	92.4*	83.0*	64.6*	49.3*
250	84.6*	78.4*	49.0*	39.5*
200	71.8*	60.0*	23.0*	15.0*
150	54.0*	44.2*	11.5*	4.0
100	33.2*	22.1*	2.6	0
50	10.5*	3.8	0	0
ED_{95} (μg/ml)	338.80	422.08	546.35	598.15
ED_{50} (μg/ml)	127.70	153.85	257.23	315.82
Regression constants Y = mX + C				
m =	106.360	102.670	137.550	162.24
C =	-174.040	-174.550	-281.540	-355.51
r =	0.990	0.993	0.987	0.9[illegible]

* Data used for regression analysis

Experiment - 49

Antifungal activity of BD-29 against *H. oryzae*

(growth inhibition method)

Conc. of the compound (μg/ml)	Percentage of inhibition over control after			
	24 hrs	48 hrs	72 hrs	96 hrs
400	100	94.3*	89.6*	83.0*
300	93.4*	85.3*	81.9*	77.2*
250	86.2*	79.6*	75.0*	68.2*
200	75.8*	67.9*	62.6*	55.0*
100	64.0*	51.6*	42.9*	29.4*
50	47.9*	26.0*	15.3*	3.1
25	24.6*	3.9	0	0
ED_{95} (μg/ml)	373.76	424.24	426.0	483.90
ED_{50} (μg/ml)	59.14	96.35	127.63	165.82
Regression constants : Y = mX + C				
m =	56.20	69.90	85.97	96.75
C =	-49.58	-88.67	-131.05	-164.75
r =	0.988	0.988	0.995	0.995

* Data used for regression analysis.

Experiment- 50

Effect of Cl-6 on the growth of *Pyricularia oryzae*

Concentration (µg/ml)	Percentage of inhibition over control after			
	48 hours	72 hours	96 hours	120 hours
12	100	100	100	100
6	100	100	89.2*	84.2*
3	93.3*	80.5*	72.6*	64.0*
1.5	74.5*	68.3*	55.6*	43.8*
0.75	55.6*	48.5*	38.1*	23.7*
0.375	35.8*	26.6*	15.3*	5.04
ED_{95} (µg/ml)	3.18	4.71	6.96	8.71
ED_{50} (µg/ml)	0.61	0.84	1.27	1.85
Regression constants : Y = mX + C				
m =	62.638	60.299	61.033	66.924
C =	63.503	54.437	43.558	32.044
r =	0.999	0.998	0.999	0.999

*Data used for regression analysis.

Experiment - 51

Antifungal activity of Cl-10 against *P. oryzae* (growth inhibition method)

Conc. of the compound (μg/ml)	Percentage of inhibition over control after			
	48 hrs.	72 hrs	96 hrs	120 hrs.
12.0	100	100	100	91.95*
6.0	100	100	88.75*	75.00*
3.0	95.50*	86.75*	78.50*	58.75*
1.5	85.25*	74.35*	63.20*	42.00*
0.75	70.00*	56.00*	44.75*	22.50*
0.375	50.75*	38.75*	24.35*	12.35*
ED_{95} (μg/ml)	2.63	3.99	6.94	14.03
ED_{50} (μg/ml)	0.33	0.58	0.98	2.08
Regression constants : $Y = mX + C$				
m =	49.66	53.94	54.10	54.32
C =	74.10	62.59	50.33	32.69
r =	0.988	0.997	0.992	0.997

* Data used for regression analysis.

Experiment -52

Effect of Cl-24 on the growth of *P. oryzae*

Concentration (µg/mL)	Percentage of inhibition over control after			
	48 hours	72 hours	96 hours	120 hours
40	100	100	93.18 *	88.33 *
20	100	94.54*	83.33 *	70.45 *
10	79.54 *	72.73*	71.67 *	59.09 *
5	63.63*	61.66*	58.87*	52.24 *
2.5	54.54*	43.43*	41.67*	35.41 *
1.25	45.45 *	36.36 *	31.54 *	23.33 *
ED_{95} (µg/ml)	29.7342	24.3360	39.4988	65.8541
ED_{50} (µg/ml)	1.8062	2.8592	3.4155	5.4143
Regression constants : Y = mX + C				
m =	36.9916	48.3872	42.3280	41.4730
C =	40.5019	27.9233	27.4198	19.5777
r =	0.9889	0.9874	0.9967	0.9941

*Data used for regression analysis.

Experiment - 53

Antifungal activity of Bp-6 against *P. oryzae*
(growth inhibition method)

Conc. of the compound (μg/ml)	Percentage of inhibition over control after			
	48 hrs.	72 hrs	96 hrs.	120 hrs.
12	100.00	100.00	100.00	100.00
6	100.00	100.00	100.00	93.40*
3	100.00	96.20*	88.60*	82.00*
1.5	93.50*	84.00*	72.50*	62.60*
0.75	78.00*	65.40*	51.60*	43.20*
0.375	54.40*	48.00*	42.30*	36.00*
ED_{95} = (μg/ml)	1.51	2.31	4.11	6.23
ED_{50} = (μg/ml)	0.30	0.41	0.58	0.82
Regression constants : Y = mX + C				
m =	64.95	59.80	53.09	51.03
C =	83.42	73.27	62.39	54.46
r =	0.99	0.99	0.99	0.99

*Data used for regression analysis.

Antifungal activity of BR-10 against P. oryzae (growth inhibition method)

Experiment-54

Conc. of the compound (µg/ml)	Percentage of inhibition over control after			
	48 hrs	72 hrs	96 hrs	120 hrs
12	100.00	100.00	90.30*	80.40*
6	100.00	86.60*	80.00*	68.60*
3	88.30*	72.60*	66.40*	56.90*
1.5	72.90*	58.90*	52.20*	41.50*
0.75	53.50*	38.70*	32.60*	21.50*
0.375	34.50*	19.30*	11.30*	8.70
ED_{95} = (µg/ml)	3.72	7.70	9.85	21.04
ED_{50} = (µg/ml)	0.66	1.21	1.59	2.53
Regression constants : Y = mX + C				
m =	60.06	55.98	56.87	48.90
C =	60.76	45.36	38.49	30.30
r =	0.99	0.99	0.99	0.99

* Data used for regression analysis.

Experiment - 55

Effect of BR-24 on the growth of *Pyricularia oryzae* (growth inhibition method)

Concentration	Percentage of inhibition over control after			
	48 hours	72 hours	96 hours	120 hours
50	100	90.83	81.82	72.73
25	81.82	78.33	68.33	54.54
12.5	72.92	61.66	54.54	43.33
5	63.71	56.25	45.45	33.33
2.5	51.61	41.79	36.67	23.95
1.25	38.71	29.17	23.95	18.66
ED_{95} (μg/ml) =	58.0534	69.5347	143.0292	358.8513
ED_{50} (μg/ml) =	2.3710	4.4737	7.0103	14.8522
Regression constants : Y = mX + C				
m =	32.4001	37.7665	34.3594	32.5351
C =	37.8518	25.4265	20.9409	11.8756
r =	0.9921	0.9901	0.9937	0.9821

* Data used for regression analysis.

Experiment - 56

Effect of Bp-27 on the growth of *Pyricularia oryzae*

Concentration (µg/ml)	Percentage of inhibition over control after			
	48 hours	72 hours	96 hours	120 hours
50	100	88.33 *	71.64 *	65.83 *
25	81.82 *	75.83 *	62.50 *	54.54 *
12.5	72.73 *	63.63 *	52.24 *	41.67 *
5	63.54 *	56.25 *	41.79 *	35.82 *
2.5	52.27 *	43.33 *	36.36 *	28.35 *
1.25	38.33 *	31.34 *	30.00 *	26.12 *
ED_{95} (µg/ml) =	58.2890	85.6927	458.0207	1092.6083
ED_{50} (µg/ml) =	2.3713	4.0770	8.5692	16.5631
Regression constants = Y = mX + C				
m =	32.3612	34.0238	26.0425	24.7345
C =	37.8645	29.2339	25.7039	19.8451
r =	0.9910	0.9946	0.9942	0.9737

* Data used for regression analysis.

Experiment - 57

Antifungal activity of BD-1 against *P. oryzae* (growth inhibition method)

Conc. of the compound (µg/ml)	Percentage of inhibition over control after 48 hrs	72 hrs
500	72.41 *	63.26 *
400	62.05 *	54.08 *
300	53.44 *	44.89 *
250	44.82 *	40.50 *
200	36.50 *	26.53 *
150	27.50 *	18.36 *
100	17.24 *	14.28 *
50	13.79 *	10.20 *
ED_{95} = (µg/ml)	977.23	1122.01
ED_{50} = (µg/ml)	269.15	338.84
Regression constants : Y = mX + C		
m =	80.328	86.680
C =	-145.87	-169.942
r =	0.995	0.992

* Data used for regression analysis.

Experiment - 58

Antifungal activity of BD-5 against *P. oryzae* (growth inhibition method)

Conc. of the compound (μg/ml)	Percentage of inhibition over control after	
	48 hrs	72 hrs
2000	100	100
1600	100	100
1200	92*	81*
1000	73*	63*
800	51*	45*
600	24*	16*
ED_{95} = (μg/ml)	1230.26	1380.38
ED_{50} = (μg/ml)	776.24	831.76
Regression constants : Y = mX + C		
m =	224.361	212.475
C =	-598.500	-572.366
r =	0.998	0.998

* Data used for regression analysis.

Experiment - 59

Antifungal activity of BD-9 against P. oryzae (growth inhibition method)

Conc. of the compound (μg/ml)	% of inhibition over control after	
	48 h	72h
500	78.90*	68.50*
400	68.74*	59.61*
300	58.06*	47.50*
250	49.75*	38.46*
200	39.15*	30.75*
150	26.25*	17.07*
100	10.80*	8.75
50	6.65	5.50
ED_{95} = (μg/ml)	707.94	891.25
ED_{50} = (μg/ml)	251.18	316.22
Regression constants : Y = mX + C		
m =	99.177	98.533
C =	-188.096	-196.445
r =	0.999	0.999

* Data used for regression analysis.

Experiment - 60

Effect of BD-25 on the growth of *P. oryzae*

Conc. of the compound (μ g/ml)	% of inhibition over control after			
	48 hrs	72 hrs	96 hrs	120 hrs
200	100	100	100	100
150	100	100	100	96.6
100	100	97.7	86.4	72.2
50	96.5	80.0	74.8	58.7
25	82.4	68.3	58.3	51.4
12.5	55.0	49.4	39.0	28.5
5.0	34.5	26.6	12.8	4.7
ED_{95} (μ g/ml)	41.78	85.94	122.63	146.29
ED_{50} (μ g/ml)	9.03	12.60	20.11	24.13
Regression Constants: $Y = mX + C$				
m =	67.636	53.970	57.320	57.500
C =	-14.642	-9.390	-24.720	-29.500
r =	0.996	0.995	0.995	0.998

Experiment - 61

Effect of BD-29 on the growth of *P. oryzae*

Concen. of the compound (μg/ml)	% of inhibition over control after			
	48 hrs	72 hrs	96 hrs	120 hrs
200	100	100	100	100
150	100	100	100	90.5
100	100	98.8	96.0	82.0
50	99.5	84.2	78.2	63.5
25	73.2	66.4	60.0	45.2
12.5	54.6	49.5	43.5	29.4
5.0	32.2	27.2	15.5	8.2
ED_{95} (μg/ml)	59.63	78.24	89.71	183.53
ED_{50} (μg/ml)	10.15	12.65	16.55	28.13
Regression Constants : $Y = mX + C$				
m =	58.58	56.88	61.52	55.25
C =	-8.92	-12.70	-25.14	-30.07
r =	0.999	0.999	0.994	0.998

Experiment-62

Chemical Hydrolysis of Cl-6

(pH 11.85, 0.0095(M) NaOH (in 50% ethanol), Initial concentration $C_o = 5.7519 \times 10^{-4}$(M)
Eempterature = 30°C)

λ (nm)	Time (hour)	O.D.	C_t(M)	$\log \frac{C_o}{C_o - C_t}$	K_{hyd} (min^{-1})	Average K_{hyd} (min^{-1})	$T_{1/2}$(hr.)
	0	0.19	-	-			
294	240	0.31	0.46×10^{-4}	0.03620	0.579×10^{-5}		
	0	0.08	-	-			
298	240	0.22	0.451×10^{-4}	0.0355	0.567×10^{-5}	0.567×10^{-5}	2021
	0	0.06	-	-			
300	240	0.21	0.442×10^{-4}	0.03472	0.555×10^{-5}		

Experiment-63

Chemical hydrolysis of compound Cl-10 pH 11.85, 0.0095(M) NaOH (in 50% ethanol. Initial concentration $C_o = 7.5063 \times 10^{-4}$(M). Temperature= 30°C

λ (nm)	Time (hrs)	O.D.	$C_t \times 10^{-4}$(M)	Log $\frac{C_o}{C_o - C_t}$	$10^6 K_{hyd}$ (min^{-1})	$10^6 K_{hyd}$ (min^{-1}) (Average)	$T_{\frac{1}{2}}$ (hrs)
	0	0.79					
292	240	0.94	0.903	0.055	8.91		
	0	0.56					
294	240	0.76	0.901	0.056	8.86		
	0	0.32				8.85	1305
296	240	0.57	0.901	0.056	8.86		
	0	0.17					
298	240	0.45	0.89	0.054	8.77		

Experiment - 64

Chemical hydrolysis of Cl-24 in 9.5 mM NaOH in 50% Ethanol (pH 11.85) at 30°C

λ = 294 nm; ϵ (for chloro saligenin) = 3069; $C_o = 1.4408 \times 10^{-4}$ (M)

Sl. No.	Time (min)	O.D.	$C_t \times 10^4$ (M)	$\log \frac{C_o}{C_o - C_t}$	K_{hyd} (min^{-1})
1.	2	0.003	0.0098	0.0029	
2.	5	0.008	0.0261	0.0079	
3.	8	0.018	0.0586	0.0180	
4.	10	0.028	0.0912	0.0284	
5.	12	0.031	0.1010	0.0316	6.2181×10^{-3}
6.	15	0.037	0.1205	0.0379	
7.	20	0.051	0.1662	0.0532	
8.	25	0.058	0.1890	0.0610	
9.	30	0.073	0.2378	0.0783	
10.	35	0.084	0.2737	0.0915	
11.	40	0.095	0.3095	0.1050	
12.	45	0.106	0.3454	0.1190	

Regression constants : $Y = mX + C$

$m = 0.0027$

$C = -0.0024$

$r = 0.9985$

Experiment-65

Chemical hydrolysis of Cl-24 in 9.5 mM NaOH in 50% Ethanol (pH 11.85) at 30°C

λ = 298 nm; ϵ (for chloro saligenin) = 3483; $C_o = 1.4408 \times 10^{-4}$ (M)

Sl. No.	Time (min)	O.D.	$C_t \times 10^4$ (M)	$\log \frac{C_o}{C_o - C_t}$	K_{hyd} (min^{-1})
1.	2	0.002	0.0057	0.0017	
2.	5	0.010	0.0287	0.0087	
3.	8	0.019	0.0545	0.0167	
4.	10	0.022	0.0631	0.0195	5.7575×10^{-3}
5.	12	0.031	0.0890	0.0277	
6.	15	0.040	0.1148	0.0360	
7.	20	0.054	0.1550	0.0494	
8.	25	0.066	0.1895	0.0612	
9.	30	0.080	0.2297	0.0754	
10.	35	0.087	0.2498	0.0827	
11	40	0.094	0.2699	0.0901	
12.	45	0.114	0.3273	0.1119	

Regression constants : $Y = mX + C$

$m = 0.0025$

$C = -0.0028$

$r = 0.9969$

Experiment - 66

Chemical hydrolysis of CL-24 in 9.5 mM NaOH in 50% ethanol (pH 11.85) at 30°C

λ = 300 nm; ϵ (for chloro saligenin) = 3655; $C_o = 1.4408 \times 10^{-4}$ (M)

Sl. No.	Time (min)	O.D.	$C_t \times 10^4$ (M)	$\log \frac{C_o}{C_o - C_t}$	K_{hyd} (min^{-1})
1.	2	0.004	0.0109	0.0033	
2.	5	0.009	0.0246	0.0075	
3.	8	0.017	0.0465	0.0142	
4.	10	0.031	0.0848	0.0263	5.7575×10^{-3}
5.	12	0.034	0.0930	0.0290	
6.	15	0.041	0.1121	0.0352	
7.	20	0.058	0.1587	0.0507	
8.	25	0.070	0.1915	0.0619	
9.	30	0.079	0.2161	0.0706	
10.	35	0.090	0.2462	0.0814	
11.	40	0.107	0.2927	0.0986	
12.	45	0.119	0.3256	0.1112	

Regression constants : $Y = mX + C$

$m = 0.0025$

$C = -0.0023$

$r = 0.9976$

Experiment - 67

Chemical hydrolysis of compound Bp-6. pH 11.85, 0.0095(M) NaOH (in 50% ethanol). Initial concentration $C_o = 0.994 \times 10^{-4}$(M). Temperature 30°C.

λ (nm)	Time (hour)	O.D.	C_t(M)	$\log \frac{C_o}{C_o - C_t}$	K_{hyd} (min^{-1})	Average K_{hyd} (min^{-1})	$T_{½}$(hr.)
	0	0.040	-	-			
294	240	0.238	0.882×10^{-4}	0.948	1.515×10^{-4}		
	0	0.050	-	-			
298	240	0.270	0.877×10^{-4}	0.929	1.485×10^{-4}	1.489×10^{-4}	77.5
	0	0.050	-	-			
300	240	0.280	0.874×10^{-4}	0.918	1.467×10^{-4}		

Experiment - 68

Chemical hydrolysis of Compound BR-10. pH 11.85, 0.0095(M) NaOH (in 50% ethanol). Initial concentration, $C_o = 3.671 \times 10^{-4}$(M), Temperature 30°C.

λ (nm)	Time (hour)	O.D.	C_t(M)	Log $\frac{C_o}{C_o - C_t}$	K_{hyd} (min^{-1})	Average K_{hyd} (min^{-1})	$T_{½}$(hr.)
	0	0.19	-	-			
294	240	0.38	0.892×10^{-4}	0.120	1.91×10^{-5}		
	0	0.11	-	-			
298	240	0.319	0.771×10^{-4}	0.102	1.63×10^{-5}	1.72×10^{-5}	670
	0	0.101	-	-			
300	240	0.322	0.772×10^{-4}	0.102	1.63×10^{-5}		

Experiment - 69

Chemical hydrolysis of BR-24 in 9.5 mM NaOH in 50% Ethanol (pH 11.85) at 30°C

λ = 294 nm ; ϵ (for Bromo Saligenin) = 2646; $C_o = 6.7164 \times 10^{-4}$ (M)

Sl. No.	Time min (x)	O.D.	$C_t \times 10^4$ (M)	Log $\frac{C_o}{C_o - C_t}$ (Y)	K_{hyd} (min^{-1})
1.	2	0.054	0.2041	0.0134	
2.	5	0.068	0.2570	0.0169	
3.	8	0.080	0.3023	0.0200	
4.	10	0.088	0.3326	0.0220	6.9090×10^{-3}
5.	12	0.140	0.5291	0.0356	
6.	15	0.175	0.6614	0.0450	
7.	20	0.251	0.9486	0.0661	
8.	25	0.298	1.1262	0.0797	
9.	30	0.314	1.1866	0.0844	
10.	35	0.380	1.4361	0.1045	
11.	40	0.440	1.6629	0.1235	
12.	45	0.485	1.8329	0.1384	

Regression constants : $Y = mX + C$

$m = 0.0030$
$C = 0.0018$
$r = 0.9944$

Experiment - 70

Chemical hydrolysis of BP-24 in 9.5 mM NaOH in 50% Ethanol (pH 11.85) at 30°C

λ = 298 nm; ϵ (for Bromo saligenin) = 3009; $C_o = 6.7164 \times 10^{-4}$ (M)

Sl. No.	Time (min)	O.D.	$C_t \times 10^4$ (M)	Log $\frac{C_o}{C_o - C_t}$	K_{hyd} (min^{-1})
1.	2	0.038	0.1263	0.0082	
2.	5	0.049	0.1628	0.0106	
3.	8	0.059	0.1961	0.0129	
4.	10	0.066	0.2193	0.0144	
5.	12	0.149	0.4952	0.0332	7.3696×10^{-3}
6.	15	0.209	0.6946	0.0471	
7.	20	0.270	0.8973	0.0623	
8.	25	0.334	1.1100	0.0784	
9.	30	0.375	1.2462	0.0891	
10.	35	0.436	1.4490	0.1055	
11.	40	0.464	1.5420	0.1133	
12.	45	0.559	1.8577	0.1406	

Regression constants : $Y = mX + C$

$m = 0.0032$

$C = -0.0054$

$r = 0.9916$

Experiment-7/

Chemical hydrolysis of BG-24 in 9.5 mM NaOH in 50% Ethanol (pH 11.85) at 30°C

λ = 300 nm ; ϵ (for Bromo Saligenin) = 3132; $C_o = 6.7164 \times 10^{-4}$ (M)

Sl. No.	Time (min)	O.D	$C_t \times 10^4$ (M)	Log $\frac{C_o}{C_o - C_t}$	K_{hyd} (min^{-1})
1.	2	0.033	0.1053	0.0069	
2.	5	0.042	0.1341	0.0087	
3.	8	0.083	0.2650	0.0175	
4.	10	0.136	0.4342	0.0290	
5.	12	0.188	0.6002	0.0406	6.6787×10^{-3}
6.	15	0.225	0.7184	0.0491	
7.	20	0.281	0.8972	0.0623	
8.	25	0.300	0.9578	0.0668	
9.	30	0.373	1.1909	0.0848	
10.	35	0.443	1.4144	0.1027	
11.	40	0.497	1.5868	0.1170	
12.	45	0.527	1.6826	0.1252	

Regression Constants : $Y = mX + C$

$m = 0.0029$

$C = 0.0003$

$r = 0.9943$

Experiment-72

Chemical hydrolysis of BP-27 in 9.5 mM NaOH in 50% Ethanol (pH 11.85) at 30°C

λ = 294 nm; ϵ (for Bromo Saligenin) = 2646; $C_o = 6.7164 \times 10^{-4}$ (M)

Sl. No.	Time (min)	O.D.	$C_t \times 10^4$ (M)	$\log \frac{C_o}{C_o - C_t}$ (Y)	K_{hyd} (min^{-1})
1.	2	0.032	0.1209	0.0098	
2.	5	0.049	0.1852	0.0152	
3.	8	0.085	0.3212	0.0267	
4.	10	0.122	0.4611	0.0389	5.9878×10^{-3}
5.	12	0.126	0.4762	0.0402	
6.	15	0.148	0.5593	0.0476	
7.	20	0.178	0.6727	0.0579	
8.	25	0.193	0.7294	0.0632	
9.	30	0.216	0.8163	0.0714	
10.	35	0.290	1.0960	0.0988	
11.	40	0.325	1.2283	0.1124	
12.	45	0.360	1.3605	0.1265	

Regression constants : $Y = mX + C$

$m = 0.0026$

$C = 0.0057$

$r = 0.9897$

Experiment - 73

Chemical hydrolysis of BP-27 in 9.5 mM NaOH in 50% Ethanol (pH 11.85) at 30°C

λ = 298 nm; ϵ (for Bromo Saligenin) = 3009; $C_o = 5.3846 \times 10^{-4}$ (M)

Sl. No.	Time (min)	O.D	$C_t \times 10^4$ (M)	$\log \frac{C_o}{C_o - C_t}$	K_{hyd} (min^{-1})
1.	2	0.029	0.0964	0.0078	
2.	5	0.037	0.1229	0.0100	
3.	8	0.057	0.1894	0.0155	
4.	10	0.126	0.4187	0.0351	
5.	12	0.130	0.4320	0.0363	5.3878×10^{-3}
6.	15	0.152	0.5051	0.0428	
7.	20	0.209	0.6946	0.0600	
8.	25	0.236	0.7843	0.0684	
9.	30	0.276	0.9172	0.0811	
10.	35	0.301	1.0003	0.0892	
11.	40	0.339	1.1266	0.1019	
12.	45	0.402	1.3360	0.1238	

Regression constants : $Y = mX + C$

$m = 0.0026$

$C = 0.0021$

$r = 0.9926$

Experiment - 74

Chemical hydrolysis of BP-27 in 9.5 mM NaOH in 50% Ethanol (pH 11.85) at 30°C

λ = 300 nm; t (for Bromo saligenin) = 3132; $C_o = 5.3846 \times 10^{-4}$ (M)

Sl. No.	Time (min)	O.D.	$C_t \times 10^4$ (M)	$\log \frac{C_o}{C_o - C_t}$	K_{hyd} (min^{-1})
1.	2	0.024	0.0766	0.0062	
2.	5	0.034	0.1085	0.0088	
3.	8	0.044	0.1405	0.0115	
4.	10	0.079	0.2522	0.0208	5.5272×10^{-3}
5.	12	0.131	0.4182	0.0351	
6.	15	0.171	0.5460	0.0464	
7.	20	0.215	0.6864	0.0592	
8.	25	0.243	0.7758	0.0676	
9.	30	0.274	0.8748	0.0770	
10.	35	0.283	0.9036	0.0798	
11.	40	0.300	0.9578	0.0851	
12.	45	0.388	1.2388	0.1135	

Regression constants : Y = mX + C

m = 0.0024

C = 0.0017

r = 0.9783

Experiment - 75

Hydrolysis of BD-1 in phosphate buffer, pH 7.7 at 30°C

[λ = 400 nm; ϵ (for 5-nitro saligenin) = 14583; Initial conc. of the compound = 5.93×10^{-5} (M)]

No.	t(min) (X)	O.D.	log $\frac{C_o}{C_o - C_t}$ (Y)	K_{hyd} (min^{-1})
1.	2	0.022	0.0113	
2.	7	0.060	0.0314	
3	12	0.108	0.0579	
4	17	0.136	0.0747	
5.	22	0.174	0.0975	4.065×10^{-3}
6.	27	0.207	0.1192	
7.	32	0.230	0.1343	
8.	37.5	0.260	0.1553	
9.	42	0.285	0.1736	
10	47	0.320	0.2007	
11.	52	0.340	0.2169	
12.	57	0.355	0.2295	
13.	62	0.385	0.2558	
14.	67	0.415	0.2839	

Regression equn. with regression constants:

$Y = mX + C$

$m = 4.065 \times 10^{-3}$

$C = 0.0055$

$r = 0.999$

Experiment - 76

Hydrolysis of BD-1 in phosphate buffer; pH 7.7 at 30°C

[λ = 410 nm; ϵ (for 6-nitro saligenin) = 15451; Initial conc. of the compound = 5.93×10^{-5} (M)]

No.	t(min) (X)	O.D.	$\log \frac{C_o}{C_o - C_t}$ (Y)	K_{hyd} (min^{-1})
1.	3	0.036	0.0175	
2.	8	0.070	0.0348	
3.	13	0.125	0.0636	
4.	18	0.155	0.0804	
5.	23	0.197	0.1052	
6.	28	0.222	0.1204	4.056×10^{-3}
7.	33	0.250	0.1383	
8.	38	0.285	0.1618	
9.	43	0.315	0.1829	
10.	48	0.330	0.1939	
11.	53	0.370	0.2246	
12.	58	0.390	0.2408	
13.	63	0.420	0.2663	
14.	68	0.430	0.2933	

Regression constants:

$Y = mX + C$

$m = 4.056 \times 10^{-3}$

$C = 0.0061$

$r = 0.996$

Experiment - 77

Hydrolysis of BD-1 in phosphate buffer, pH 7.7 at 30°C

[λ = 420 nm; ϵ (for 5-nitro saligenin) = 14757; Initial conc. of the compound = 5.93×10^{-5} (M)]

No.	t(min.) (X)	O.D.	log $\frac{C_o}{C_o - C_t}$ (Y)	K_{hyd} (min^{-1})
1.	4	0.041	0.0208	
2.	9	0.076	0.0393	
3.	14	0.125	0.0669	
4.	19	0.155	0.0846	
5.	24	0.194	0.1087	
6.	29	0.225	0.1291	4.158×10^{-3}
7.	34	0.255	0.1495	
8.	39	0.275	0.1638	
9.	44	0.310	0.1899	
10.	49	0.335	0.2095	
11.	54	0.370	0.2386	
12.	59	0.375	0.2430	
13.	64	0.400	0.2652	
14.	69	0.435	0.2985	

Regression constants:

$Y = mX + C$

$m = 4.158 \times 10^{-3}$

$C = 0.0059$

$r = 0.998$

Experiment -78

Hydrolysis of BD-1 in phosphate buffer, pH 7.7 at 30°C

[λ = 430 nm; ϵ (for 5-nitro saligenin) = 12326; Initial conc. of the compound = 5.93×10^{-5} (M)]

No.	t(min) (X)	O.D.	log $\frac{C_o}{C_o - C_t}$ (Y)	K_{hyd} (min^{-1})
1.	5	0.036	0.0220	
2.	10	0.065	0.0407	
3.	15	0.108	0.0693	
4.	20	0.139	0.0920	
5.	25	0.164	0.1105	
6.	30	0.194	0.1338	
7.	36.5	0.225	0.1601	4.221×10^{-3}
8.	40	0.240	0.1728	
9.	45	0.260	0.1909	
10.	50	0.280	0.2097	
11.	55	0.295	0.2195	
12.	60	0.320	0.2501	
13.	65	0.340	0.2717	
14.	70	0.380	0.3186	

Regression constants

$Y = mX + C$

$m = 4.221 \times 10^{-3}$

$r = 0.995$

$c = 0.0028$

Experiment - 79

Hydrolysis of BD-5 in Phosphate buffer, pH 7.7 at 30°C

[λ = 400 nm, ∈ (for 5-nitro saligenin) = 14583; Initial conc. of the compound = 5.19×10^{-5} (M)]

No.	t(min) (X)	O.D.	log $\frac{C_o}{C_o - C_t}$ (Y)	K_{hyd} (min^{-1})
1.	3	0.015	0.0089	
2.	8	0.027	0.0157	
3.	18	0.046	0.0271	
4.	33	0.060	0.0361	
5.	53	0.076	0.0458	
6.	86	0.136	0.0865	1.911×10^{-3}
7.	123	0.155	0.0994	
8.	143	0.194	0.1284	
9.	201	0.250	0.1741	
10.	242	0.290	0.2096	
11.	255	0.300	0.2192	
12.	270	0.315	0.2337	

Rregression Constants : $Y = mX + C$

$m = 8.300 \times 10^{-4}$

$C = 0.0078$

$r = 0.993$

Experiment No.- 80

Hydrolysis of BD-5 in Phosphate buffer, pH 7.7 at 30°C

[λ = 410 nm; ϵ (for 5-nitro saligenin = 15451; Initial conc. of the compound = 5.19×10^{-5} (M)]

No.	t(min) (X)	O.D.	log $\frac{C_o}{C_o - C_t}$ (Y)	K_{hyd} (min^{-1})
1	4	0.017	0.0097	
2	9	0.031	0.0174	
3.	20	0.046	0.0255	
4.	34	0.065	0.0370	
5.	54	0.083	0.0477	
6.	88	0.142	0.0851	1.962×10^{-3}
7.	124	0.167	0.1017	
8.	144	0.215	0.1355	
9.	202	0.270	0.1783	
10.	243	0.310	0.2122	
11.	256	0.325	0.2256	
12.	271	0.340	0.2395	

Regression Constants : $Y = mX + C$

$m = 8.519 \times 10^{-4}$

$C = 0.0068$

$r = 0.998$

Experiment - 81

Hydrolysis of BD-5 in Phosphate buffer; pH 7.7 at 30°C

[λ = 420 nm; ϵ (for 5-nitro saligenin) = 14757; Initial conc. of the compound = 5.19×10^{-5} (M)]

No.	t(min) (X)	O.D.	$\log \frac{C_o}{C_o - C_t}$ (Y)	K_{hyd} (min^{-1})
1.	5	0.018	0.0102	
2.	10	0.031	0.0182	
3.	22	0.050	0.0297	
4.	35	0.065	0.0388	
5.	57	0.083	0.0501	
6.	89	0.139	0.0874	1.953×10^{-3}
7.	125	0.155	0.0981	
8.	145	0.197	0.1293	
9.	203	0.260	0.1801	
10.	244	0.300	0.2158	
11.	257	0.310	0.2253	
12.	272	0.325	0.2398	

Regression Constants : $Y = mX + C$

$m = 8.482 \times 10^{-4}$

$C = 0.0067$

$r = 0.998$

Experiment No.-82

Hydrolysis of BD-5 in Phosphate buffer; pH 7.7 at 30°C

[λ = 430 nm; ϵ (for 5-nitro saligenin) = 12326; Initial conc. of the compound = 5.19×10^{-5} (M)]

No.	t(min) (X)	O.D.	Log $\frac{C_o}{C_o - C_t}$ (Y)	K_{hyd} (min^{-1})
1	6	0.018	0.0122	
2	11	0.031	0.0219	
3	24	0.043	0.0305	
4	36	0.058	0.0412	
5	58	0.081	0.0587	
6	90	0.110	0.0825	1.998×10^{-3}
7	126	0.136	0.1044	
8	146	0.167	0.1318	
9	204	0.220	0.1830	
10	245	0.255	0.2208	
11	258	0.265	0.2323	
12	273	0.280	0.2500	

Regression constant : $Y = mX + C$

$m = 8.674 \times 10^{-4}$

$C = 0.0073$

$r = 0.998$

Experiment -83

Hydrolysis of BD-9 in phosphate buffer, pH 7.7 at 30°C

[λ = 400 nm; ϵ (for 5-nitro saligenin) = 14583 ; Initial conc. of the compound = 1.278×10^{-4} (M)]

No.	t(min) (X)	O.D.	log $\frac{C_o}{C_o - C_t}$ (Y)	K_{hyd} (min^{-1})
1	2	0.161	0.0392	
2	6	0.320	0.0818	
3	10	0.430	0.1138	
4	14	0.580	0.1618	$9.482 \times$
5	18	0.680	0.1970	
6	22	0.769	0.2311	
7	26	0.854	0.2660	

Regression constants : $Y = mX + C$

$m = 9.482 \times 10^{-3}$

$C = 0.023$

$r = 0.998$

Experiment - 84

Hydrolysis of BD-9 in phosphate buffer, pH 7.7 at 30°C

[λ = 410 nm; ϵ (for 5-nitro saligenin) = 15451; Initial conc. of the compound = 1.278×10^{-4} (M)]

No.	t(min) (X)	O.D.	Log $\frac{C_o}{C_o - C_t}$ (Y)	K_{hyd} (min^{-1})
1	3	0.200	0.0463	
2	7	0.375	0.0914	
3	11	0.510	0.1297	
4	15	0.640	0.1700	9.714×10^{-3}
5	19	0.750	0.2073	
6	23	0.854	0.2458	
7	27	0.939	0.2802	

Regression Constants: $Y = mX + C$

$m = 9.714 \times 10^{-3}$

$C = 0.0215$

$r = 0.999$

Experiment-85

Hydrolysis of BD-9 in phosphate buffer, pH 7.7 at 30°C

[λ = 420 nm; ϵ (for 5-nitro saligenin) = 14757; Initial conc. of the compound = 1.278×10^{-4} (M)]

No.	t(min) (X)	O.D.	log $\frac{C_o}{C_o - C_t}$ (Y)	K_{hyd} (min^{-1})
1	4	0.235	0.0577	
2	8	0.385	0.0991	
3	12	0.520	0.1400	9.857×10^{-3}
4	16	0.640	0.1799	
5	20	0.750	0.2200	
6	24	0.854	0.2616	
7	28	0.921	0.2907	

Regression constants : $Y = mX + C$

$m = 9.857 \times 10^{-3}$

$C = 0.0207$

$r = 0.999$

Experiment-86

Hydrolysis of BD-9 in phosphate buffer, pH 7.7 at 30°C

[λ = 430 nm; ϵ (for 5-nitro saligenin) = 12326; Initial conc. of the compound = 1.278×10^{-4} (M)]

No.	t(min) (X)	O.D.	log $\frac{C_o}{C_o - C_t}$ (Y)	K_{hyd} (min^{-1})
1	5	0.230	0.0685	
2	9	0.350	0.1091	
3	13	0.470	0.1538	
4	17	0.560	0.1906	9.713×10^{-3}
5	21	0.650	0.2309	
6	25	0.730	0.2702	
7	29	0.783	0.2980	

Regression constants: $Y = mX + C$

$m = 9.713 \times 10^{-3}$

$C = 0.0236$

$r = 0.998$

Experiment - 87

Chemical hydrolysis of BD-25

(pH = 11.85, 0.0095(M) NaOH (in 50% ethanol)

C_o = 5.53 x 10^{-5} (M), temperature = 30°C

λ (nm)	Molar extinction co-efficient	Time (hours)	O.D.	$C_t \times 10^{-5}$ (M)	$\log \frac{C_o}{C_o - C_t}$	$K_{hyd} \times 10^{-5}$ (min^{-1})
400	19090	240	0.451	2.36	0.242	3.86
410	20090	240	0.451	2.24	0.225	3.60
420	18180	240	0.370	2.03	0.199	3.17

Experiment - 88

Chemical hydrolysis of BD - 29

pH = 11.85, 0.0095 M NaOH

C_o = 3.10 x 10^{-5} (M)

tempt. = 30°C

λ (nm)	ϵ	Time (hours)	O.D.	$C_t \times 10^{-5}$ (M)	log $\frac{C_o}{C_o - C_t}$	K_{hyd} (min^{-1})
400	19090	240	0.271	1.41	0.2662	4.25 x 10^{-5}
410	20090	240	0.250	1.24	0.2228	3.56 x 10^{-5}
420	18180	240	0.239	1.31	0.2396	3.83 x10^{-5}