INVESTIGATION ON SOME

2-Alkylamido-6-Bromo-4H-1, 3, 2-Benzodioxaphosphorin 2-Sulphide

HAVING PESTICIDAL ACTIVITIES

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

106298

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Dedicated to
the memory of my father

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SYNOPSIS

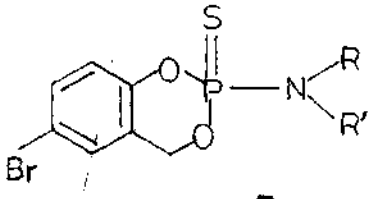
The work embodied in this dissertation is related to the investigation of some bromo saligenin cyclic phosphoramidothionates with reference to their synthesis, spectral properties, chemical, biochemical, insecticidal, fungicidal and other toxicological properties.

CHAPTER I

A general introduction of some organophosphorus pesticides has been presented. It has been reported a short review describing the chemical, biochemical, insecticidal, fungicidal and other toxicological properties of saligenin cyclic phosphorus compounds 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. Investigations have revealed that the biological activities of these compounds are greatly influenced by the exocyclic substituents on the phosphorus atom.

It was reported by Prof. Eto and his co-workers that 2-methoxy-6-nitro-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BD-6) was obtained as a paste after purification through silicic acid column chromatography and found to have about sixty times less insecticidal activity compared with salithion. However, it has been observed in this laboratory that the methoxy compound (BD-6) is a solid (m.p. 84°C) and has about 1.5 - 2 times greater oral insecticidal activity to *Periplaneta americana* than salithion and

comparable activity to grasshoppers (*Oxya nitidula*). Moreover, introduction of an amide group in place of an alkyl ester group often gives organophosphorus esters fungicidal, nematocidal and other biological activities. There are many examples in literature which show that some phosphoramidothionates, phosphoramides or phosphonamides in which the phosphorus atom is attached directly to the nitrogen atom of an amine or a hetero-cyclic compound such as phthalimide, imidazole or triazole, have very good fungicidal activity. These observation prompted us to undertake a systematic work on bromo-saligenin cyclic phosphoramidothionates. The work embodied in Chapter 2 and Chapter 3 of this dissertation is related to the investigation of some 2-alkylamido-4-bromo-4H-1,3,2-benzodioxaphosphorin 2-sulphides having general structure



where $-N\begin{matrix}R\\R^1\end{matrix}$ = Diisobutylamido

= Dipentylamido

= Dihexylamido

= Dibutylamido

= 2,6-Dimethylmorpholino

= 2-Ethylpiperidino

= 4-Benzylpiperidino

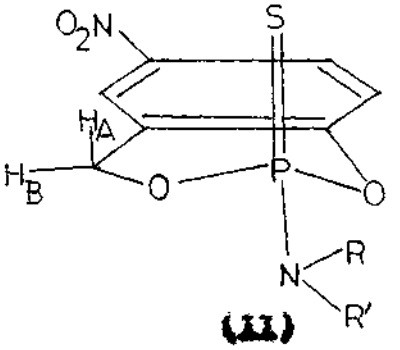
= Hexamethyleneimido.

CHAPTER 2

It deals with the work related to the synthesis and spectral properties of bromo saligenin cyclic phosphoramidothionates. The structures of these compounds have been established by chemical analysis, UV, IR, Mass, ^{1}H NMR, ^{13}C NMR spectral data. All the compounds show common IR bands : 1010 - 1040 cm^{-1}(s), P-O-C(alkyl); 1235 - 1260 cm^{-1}(s) and 870 - 910 cm^{-1}(s), P-O-C (aryl); 805 - 815 cm^{-1}, P=S(I); 640 - 690 cm^{-1}, P=S(II). In mass spectra all the compounds show molecular ion peaks (M^{+}) and $(M + 2)^{+}$ ion peaks. For the proton noise decoupled ^{13}C spectra of BR-6, BR-12, BR-14 and BR-17 at 100 MHz the saturated carbons 2 and 3 bonds from the phosphorus show splitting due to $^{13}C - {}^{31}P$ spin coupling. The chemical shift values for the CH_2 carbon (C_4) in the dioxaphosphorin ring of BR-6, BR-12, BR-14 and BR-17 are almost the same. ^{13}C chemical shifts are often quite sensitive to conformation, it supports the similarity of the structure. In ^{1}H NMR spectra all of the compounds have signals at δ = 4.63-4.74 ppm for the $-CH_2-$ group protons in the dioxaphosphorin ring.

From the study of the ^{1}H NMR spectral data of some 2-alkoxy/phenoxy/alkylamido-6-nitro-4H-1,3,2-benzodioxaphosphorin 2-sulphides it is fairly evident that the chemical shift difference of the protons H_A and H_B increases in going from 2-alkoxy to 2-alkylamido compounds, and that the 2-substituent at the same time increases in bulk, and probably spends more time in the conformation with the least steric interactions. The structure II appears

to explain, to a reasonable extent, the reversal of the expected proton chemical shift order for the quasi axial and quasi equatorial protons, due to the position of the magnetically anisotropic P=S bond relative to the $-CH_2-$group in the dioxaphosphorin ring.



(II)

CHAPTER 3

It deals with the works related to the biological activities and chemical hydrolysis of some 2-alkylamido-6-bromo-4H-1,3,2-benzodioxaphosphorin 2-sulphides.

All the compounds have less oral insecticidal activity than salithion against *P. americana*. They are less toxic to male rats than salithion and are not phytotoxic. All the bromo saligenin cyclic phosphoramidothionates show very poor anticholinesterase activity in blow-fly head homogenate and goat whole blood. From the chemical hydrolysis it has been observed that the 6-bromo saligenin cyclic phosphoramidothionates are most stable to alkaline hydrolysis (pH 11.65). These compounds show very good inhibitory effect on the growth of *Xanthomonas oryzae*; compared with that of Hinosan they have greater inhibitory effect.

A good correlation is obtained between the pED_{50} value (for *H. oryzae* at 72 hrs) and the Structural Information Content (SIC) and hydrophobic parameter (log P). The regression equation is

$$pED_{50} = \underset{(\pm 0.336)}{23.422}\ SIC - \underset{(\pm 0.232)}{27.494}\ (SIC)^2 - \underset{(\pm 0.182)}{0.096}\ \log P + \underset{(\pm 2.362)}{0.486}$$

$n = 13;\ s = 0.14;\ r = 0.94$

$F_{(3,9)}\ cal = 23.9$ and $F_{(3,9)(0.001)}\ tab = 12.9$

where n is the number of compounds, s is the standard deviation, r is the correlation coefficient, F is the statistical measure of the significance of correlation.

From the above equation it can be suggested the following

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

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

(iii) For *H. oryzae* the regression equation involving pED_{50} with SIC, $(SIC)^2$ and log P provides the best fit equation which suggests that stereo-hydrophobic make-up and topology of the bio-active molecule is a major determinant for the bioresponse.

Further studies on antifungal activity of some bromo saligenin cyclic phosphoramidothionates against *Pyricularia oryzae* have been presented.

In Chapter 4 the studies on some o-nitro/chloro saligenin cyclic phosphoramidothionates having pesticidal activities (including insecticidal activity, acute oral toxicity, anticholinesterase activity, fungicidal activity) have also been presented.

The antifungal activity data justify further examination of these phosphoramidothionates as potential fungicides. However, their application in the field to protect plants from diseases is yet 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.

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FIGURE OF DIFFERENT SPECTRA

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Common or trade names, Chemical structure and other properties of some organophosphorus compounds

APPENDIX-II

Calculations of Structural Information Content (S.I.C.)

CHAPTER 1

Chapter 1

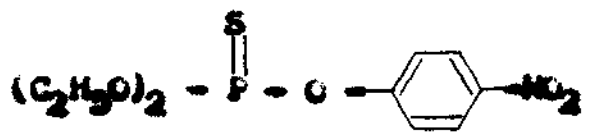
1.1 INTRODUCTION

The contributions to knowledge in the domain of pesticide chemistry have increased with such rapidity within recent years that the prospective worker in the line finds himself confronted with the vexing problem of what to exclude rather than what to include in his investigation. The pest, disease and weed situation are not static - they keep on changing. New physiological races evolve as a result of mutations in nature. Many insects and fungi develop resistant strain when the same insecticide or fungicide is used year after year. Similarly, by the use of the same herbicide, season after season there is often a shift in the weed flora and a number of resistant species become predominant. Even change in cropping pattern change the pest, disease and weed situation - many pests, diseases and weeds of minor importance may assume major proportions. It has been often noticed that pests and diseases, virtually unknown and ignored suddenly become very alarming with the introduction of new crop varieties. Bacterial blight, tungro-virus, gall, midge of paddy and Phalaris minor in wheat are only a few examples from our country.

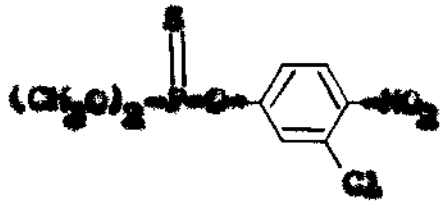
All these definitely point out that the existing pesticides, however efficient they may be cannot solve the pest problems permanently. New pesticides must be developed to combat various new situations. It should be a continuous search - a process of intensive and sustained research and development are also vital for

finding pesticides which will be safer, more effective and selective, and above all more economic in the true sense and environmentally acceptable.

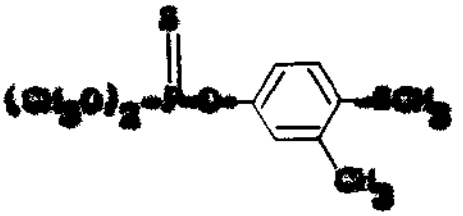
One of the most important classes of pesticidal substances is the organophosphorus compounds. Several new compounds of this group possess insecticidal, acaricidal, nematocidal, antihelmintic, insect sterilizing, fungicidal, herbicidal, rodenticidal and other properties. The systematic investigations of their fungicidal and bactericidal properties were begun after 1940[1]. 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. The development of new organophosphorus compounds was for a long time dominated almost exclusively by one single guiding principle namely the "Acyl Rule" of Schrader[2,3,4]. The great advancement in agricultural practice, scientific knowledge of the structure activity relationship and mode of action of organophosphorus pesticides were achieved by the discovery of parathion by Schrader in 1944. Parathion is extremely toxic to mammals as well as to insects. Many less toxic pesticides have synthesized by slight structural modification of parathion; for example, chlorthion (in 1932), fenthion (in 1938) and fenitrothion (in 1959) were discovered[3].



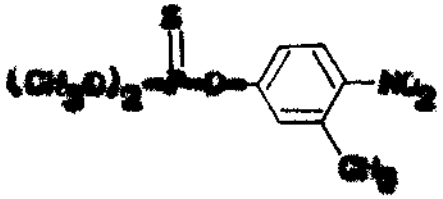
Parathion



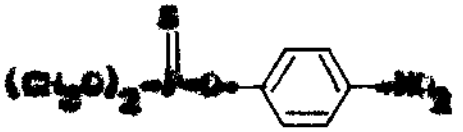
Chlorthion



Fenthion

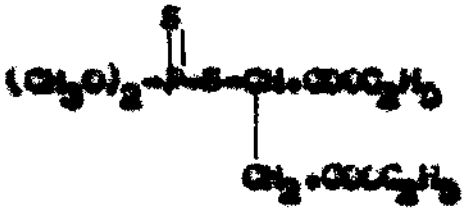


Fenitrothion



Parathion-methyl

Malathion was discovered in 1950 and demeton in 1951.



Malathion

Demeton-S

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

Common or trade names, chemical designations and other properties of some organophosphorus pesticides have been given in Appendix-I.

1.2 REVIEW ON SALITHION AND RELATED COMPOUNDS

Discovery of saligenin cyclic phosphate as a biologically active metabolite of tri-o-cresyl phosphate (TOCP)[3, 5, 6] has led to extensive studies on synthesis, chemical and biological properties of many related compounds [7, 8]. Analogous cyclic phosphorus esters have been synthetically prepared for examination of their chemical properties[9, 10] and biological activities,[11, 12] are not always coincident with the chemical reactivities and appear to be influenced by the size of an exocyclic substituent on the phosphorus atom. The TOCP-metabolite causes ataxia in hens but has no insecticidal activity, while its analogous cyclic phosphate carrying a small alkyl group have insecticidal activity [13]. Among the saligenin cyclic phosphorus compounds, salithion (2-methoxy-4H -1,3,2-benzodioxaphosphorin 2-sulphide) has been prepared in a large quantity and practically used as an insecticide in Japan [3, 8].

Organophosphorus compounds are delayed neurotoxic. A large outbreak of flaccid paralysis took place in Morocco (1959)[15] and USA (1930)[14] due to adulteration of fluid extract of ginger and cooking oil with TOCP. Aldridge and Barnes[16, 3] observed that all neurotoxic triaryl phosphate except tri-p-ethyl phenyl phosphate have at least one alkyl group carrying the -hydrogen atom on the O-position. This structure neurotoxicity relationship of triaryl phosphate is characterized by the isolation of active metabolite TOCP [5, 6]. The main active metabolite (M) is o-tolyl saligenin

cyclic phosphate (2-o-tolyl-4H-1, 3,2-benzodioxaphosphorin 2-oxide). It extraordinarily active in all the biological properties shown by TOCP. This compound (M) is about 100 times more potent to cause ataxia in hens than TOCP and also ten million times more active than TOCP in the *in vitro* inhibition of plasma cholinesterase [7].

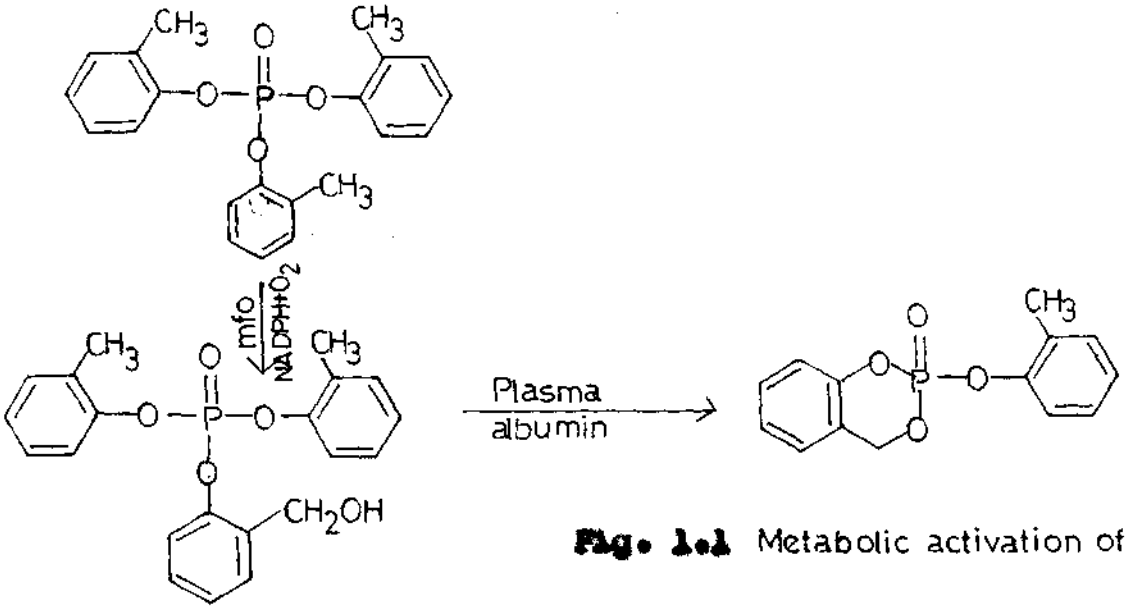
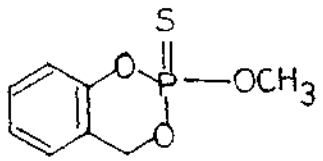


Fig. 1.1 Metabolic activation of TOCP

The triaryl phosphates having an o-alkyl group with the α-hydrogen atom may be metabolized to give the corresponding active cyclic esters. In the cyclization reaction, no alkyl ester group participates as the leaving group [18]. Actually, no aryl but alkyl saligenin cyclic phosphate is formed *in vivo* from alkyl di-o-tolyl phosphates [19]. Such metabolic activation of TOCP or its analogues have been observed in rats,[16] hens[16], cats[17] and insects [20].

All aryl saligenin cyclic phosphates have shown no insecticidal activity but manifested a high delayed neurotoxicity to cause ataxia in hens; surprisingly the corresponding cyclic esters (both X=O and X=S compounds) having a small alkyl group on phosphorus revealed high insecticidal activity [20,21].

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



Salithion

The cyclic phosphate and phosphonate esters of saligenin are synthesized by the reaction of saligenin and substituted phosphoryldichlorides in the presence of a dehydrochlorinating agent such as tertiary amine in a dry solvent e.g. chloroform, toluene at low temperature [19]. In some cases instead of tertiary amine potassium carbonate and finely divided copper powder at high temperature[21] are used. Thus salithion was first prepared in

very low yield. This difficulty has been overcome later by applying the Schotten-Baumann acylation procedure using an aqueous solution of sodium hydroxide (Fig.1.2).

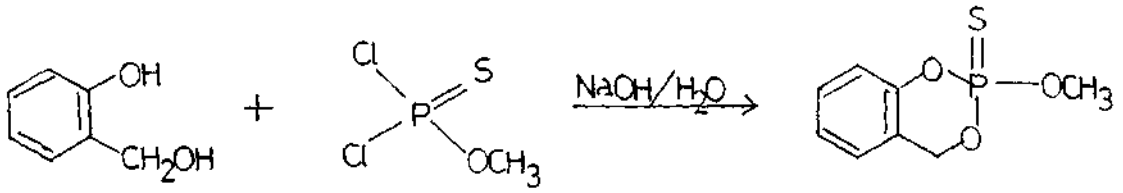
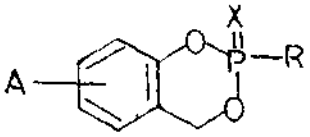


Fig.1.2 - Synthesis of Salithion

Careful observation of the literature reveals[30,31,32,33,34,35,36,37] that a variety of saligenin cyclic phosphorus esters have been prepared and examined for insecticidal activity as well as other biological properties. A list containing these type of compounds is given in table 1.2 and table 1.3.

Table 1.2

Substituted Saligenin cyclic phosphorus Esters with physical properties.



R	A	X	*Procedure	b.p. °C/mm Hg(m.p. °C)
OCH_3	H	S	(S)	55-56
OCH_3	H	O	(P)	110-2/0.05
o-n-C_3H_7	H	O	(P)	129-32/0.05
OC_2H_5	H	S	(P)	Liquid(not distilled)

/Contd....

Table 1.2 (Contd.)

R	A	X	*Procedure	b.p. °C/mm Hg(m.p.°C)
OC_6H_5	H	S	(P)	30
C_6H_5	H	S	(P)	37
CH_3	H	O	(P)	140/0.5(35°)
C_2H_5	H	O	(P)	143-9/0.3(25°)
iC_3H_7	H	S	(P)	108/0.6
OCH_3	6-CH_3	O	(P)	139-140/0.3
OC_2H_5	6-CH_3	O	(P)	122-126/0.3
OCH_3	7-CH_3	O	(P)	109/0.05
$NHCH_3$	7-CH_3	O	(P)	140-146
OCH_3	8-CH_3	O	(P)	118-120/0.5
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

Contd...

Table 1.2(Contd.)

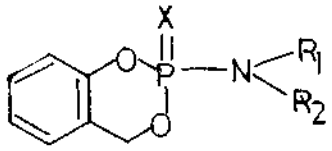
R	A	X	*Procedure	b.p.°C/mm Hg(m.p.°C)
OCH_3	6-Cl	S	(P)	170-178/0.2
$NHCH_3$	6-Cl	S	(P)	173-180/0.25
SCH_3	6-Cl	S	(P)	160-170/0.2
OCH_3	8-Cl	S	(S)	72-73
$NHCH_3$	8-Cl	S	(P)	46-47
SCH_3	8-Cl	S	(S)	Oil**
OCH_3	6-NO_2	S	(S)	Paste**
OCH_3	6-Cl, 8-C_6H_5	S	(S)	Paste*
OC_2H_5	6-Cl, 8-C_6H_5	S	(S)	Paste*
OCH_3	6-C_6H_5, 8-Cl	S	(S)	Paste*
OCH_3	6,8-Cl	S	(S)	37-38
OC_2H_5	6,8-Cl	S	(S)	Oil**
$NHCH_3$	6,8-Cl	S	(S)	Oil**
SCH_3	H	S	(S)	69-70

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

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

Table 1.3

Saligenin cyclic phosphoramidates and Phosphoramidothionates with Physical Properties



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

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

Salithion is a colourless crystal m.p. 55-56°C practically insoluble in water but easily soluble in acetone and benzene, moderately soluble in cyclohexane, toluene and xylene; vapour pressure 1.5×10^{-6} mm Hg at 250°C, UV λ_{max} nm (ϵ) 274(860), 267(800). It has characteristic IR band at 1020 cm^{-1} for P-O-CH_2 in the hetero ring. NMR $\delta(CH_2)$ ppm: 3.76(3H, doublet, J_{PH} = 14 Hz, CH_3), 5.21 (2H, doublet, J_{PH} = 15 Hz, CH_2), 6.8 - 7.2 (4H, multiplet, benzene ring). The signal at upper field of the doublet at 5.21 ppm slightly splits further (15 Hz). This becomes much significant at -20°C, suggesting the methylene protons (H_A, H_B) are not equivalent with each other, but the dioxaphosphorin ring is conformationally mobile in solution (Fig.1.3).

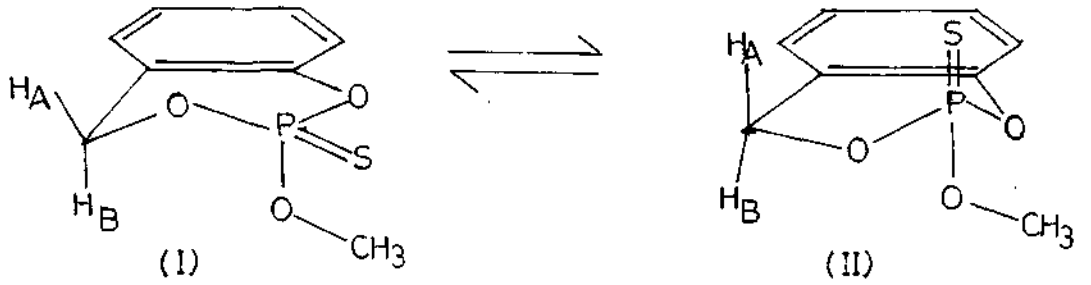


Fig. 1.3 Conformational Change of Salithion heteroring.

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

Salithion gives a characteristic modes of fragmentation in mass spectrometry[29]. It gives an intense peak of $(M-CH_3)^+$ (m/z 201) by β -cleavage occurring at the exocyclic ester group. Another fragmentation process is the direct loss of SH followed by the elimination of formaldehyde (Fig.1.4).

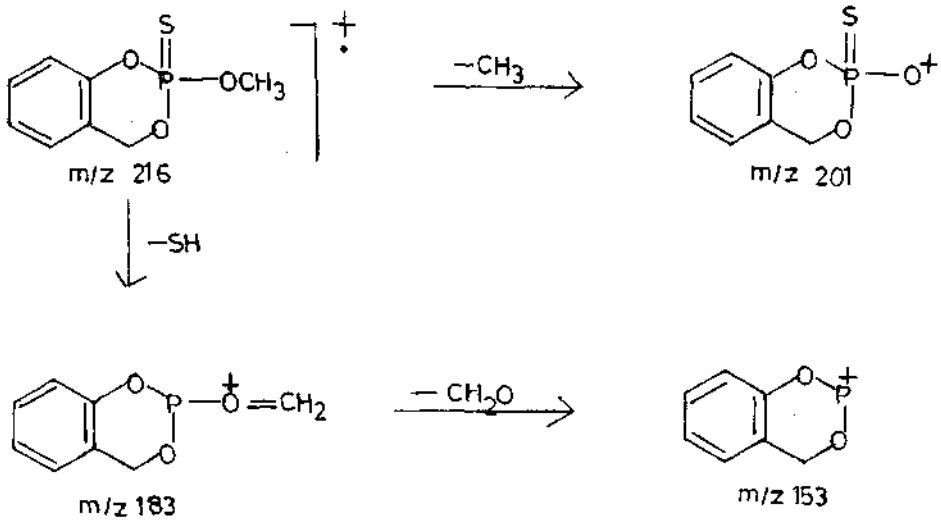


Fig. 1.4 Modes of Fragmentation of Salithion in mass spectrometry.

Salithion reacts with bromine water to give saliexon[29]. Since saliexon (2-methoxy-4H-1,3,2 benzodioxaphosphorin 2-oxide) is several thousand times more active in cholinesterase inhibition than salithion, an enzymatic method after the oxidation can be used for the residue analysis of salithion[29]. It is converted into S-alkyl saligenin cyclic phosphorothiolates by heating with alkyl iodides (the Pistenimuka reaction)[30,31] in dimethyl formamide. Saligenin is demethylated to form the salt of saligenin cyclic phosphorothionic acid by the action of certain nucleophiles

such as cyclohexylamine[28] and potassium dimethyldithiocarbamate[28,32].

Salithion is relatively unstable in storage. Some secondary amines, such as carbazole and N-phenyl α-naphthylamine, stabilized the formulation[33]. In a phosphate buffer (pH 7.7) salithion is slowly hydrolyzed by the P-O (aryl) bond cleavage[28]. The hydrolysis rate constant (25°C) is $K = 2.4 \times 10^{-4}\ min^{-1}$. Under these conditions give saligenin as the sole product. The rates of hydrolysis of the corresponding cyclic methylphosphonate, S-methylphosphorothiolate (the thiolate isomer of salithion MTBO), methyl phosphate (salioxon), and N-methyl phosphoramidate are, respectively 90,60,6 and 0.6 times greater than that of salithion. The hydrolysis of salithion is shown in Fig.1.5.

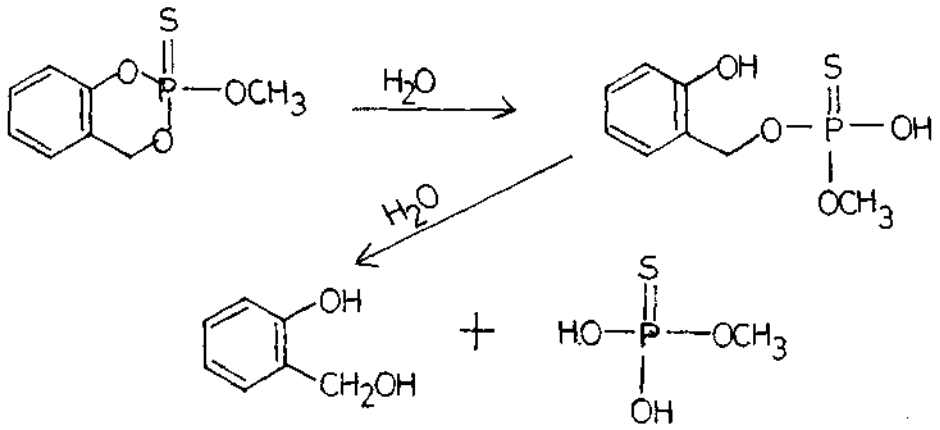


Fig. 1.5 Hydrolysis of Salithion.

Salithion is a wide-spectrum insecticide for use in orchards and vegetable gardens. It manifests the insecticidal action not only as contact and stomach poisons but also as a fumigant[34,35].

Minara et al[36] investigated the metabolism of salithion in rats and plants using ^{14}C-labelled compound.

The metabolic pathways of salithion in rats and plants have been studied[3]. These are summarised[3] in Fig. 1.6.

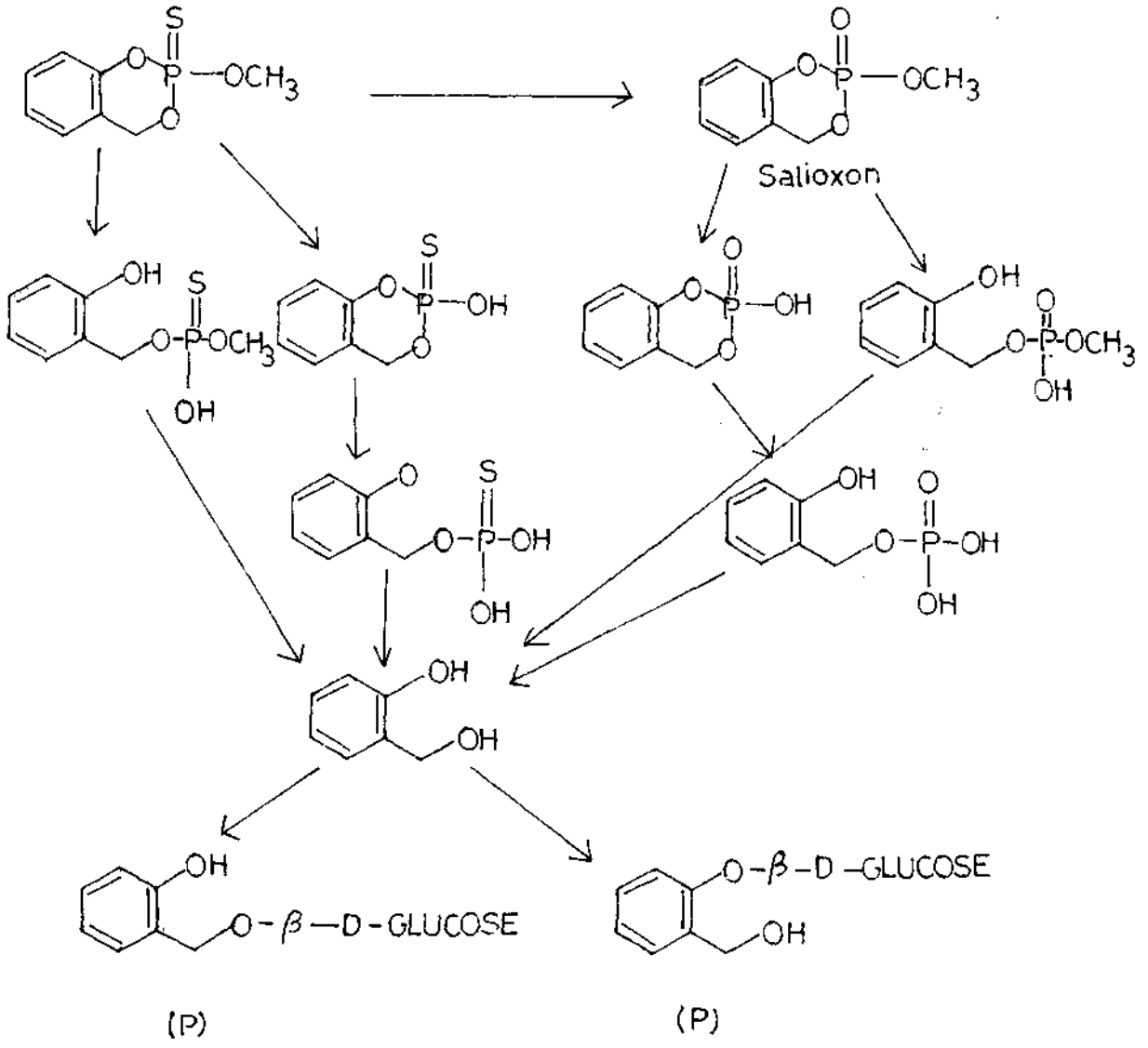
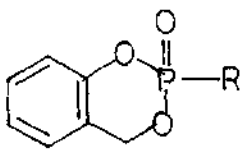


Fig.1.6 Metabolic pathways of salithion in rats and plants (P-denotes the metabolites found in plant only).

In the series of alkyl derivatives there is a relationship between hydrolysis rate constants and insecticidal activity (LD_{50}) (Table 1.4). The higher the reactivity is, the stronger the insecticidal activity. However, the aryl derivatives do not follow this relationship; they are more reactive than alkyl derivatives, but are almost non-insecticidal. The size of the substituent appears to be more important for biological activity than the electronic property of the substituent.

Table 1.4

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



R	K_{hyd} min^{-1}	LD_{50} (μg/female house fly)
CH_3	2.22×10^{-2}	0.19
C_2H_5	4.25×10^{-2}	0.17
i-C_3H_7	-	0.33
Sec-C_4H_9	-	7.0
t-C_4H_9	-	10 (0%)
$CH = CH_2$	1.39×10^{-2}	0.68
CH_2Cl	2.00×10^{-1}	10(60%)

Contd...

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Table 1.4 (Contd.)

R	k_{hyd} min^{-1}	LD_{50} (μg/female house fly)
CH_2CH_2Cl	1.41×10^{-2}	0.09
C_6H_5	1.28×10^{-2}	10 (10%)
OCH_3	1.42×10^{-2}	0.036
OC_2H_5	3.04×10^{-2}	0.33
$O\text{-}n\text{-}C_3H_7$	3.79×10^{-4}	7.1
$O\text{-}n\text{-}C_4H_9$	3.29×10^{-4}	10 (40%)
$O\text{-}CH_2CH_2Cl$	2.38×10^{-3}	0.49
$O\text{-}C_6H_5$	6.30×10^{-3}	10 (3%)
$NHCH_3$	1.54×10^{-4}	0.05
$N(CH_3)_2$	Negligibly small	0.40
NHC_6H_5	2.40×10^{-4}	10 (5%)

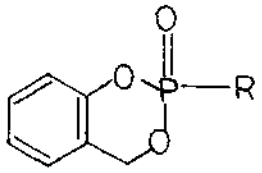
<u>Biological Activities and Structural Relationship</u> :

The saligenin cyclic phosphate esters have interesting biological activities. Some of them are neurotoxic, causing ataxia in higher animals. Others do not show such harmful activity but do high insecticidal activity, systemic activity and fungicidal activity. Their biological activities include also synergism with organophosphorus insecticides, nematocidal and antifilarial activity. The specificity in biological activi-

tion may be attributed to the steric effect of an omegyclic substituent group on the phosphorus atom as shown in table 1.5.

Table 1.5

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



R	Delayed neurotoxicity MAD[a]	Synergism with malathion acetylcholinesterase co-efficient		Insecticidal activity LD_{50}[c]
		Mice	House flies[b]	
OCH_3-C_6H_4O	2-6	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	0.38
CH_3O	N.A.[e]	3.7	4.7	0.04
$(CH_3)_2N$	N.A.[e]	1.1	-	0.30

a. Minimum ataxia dose for hens in mg/kg

b. A resistant strain

c. 50% lethal dose by topical application to housefliee in μg.

d. Percentage mortality at 10 μg/fly

e. No ataxia signs evident with any sublethal dosages.

All aryl saligenin cyclic phosphates manifest a high delayed neurotoxicity to cause ataxia in hens and high synergistic activity with malathion[12,87]. The arylphosphonate analogues showed similar biological activities but less in the neurotoxicity. On the other hand, the corresponding cyclic esters having a small alkyl group on phosphorus, i.e. 2-alkyl, 2-alkoxy and 2-alkylamido-4H-1,3,2-benzodioxaphosphorin 2-oxides, did not cause ataxia in hens with any sublethal doses and only weakly potentiated the toxicity of malathion[12]. The interesting feature is that the alkyl derivatives reveal high insecticidal activity, whereas, the aryl cyclic esters do not[11].

The specificity of saligenin cyclic phosphates in the biological activity relates to their selectivity in enzyme inhibition. These phosphates inhibit various serine enzyme by phosphorylation, producing probably salicylosyphosphinylenzymes[16,17]. This involves by opening of the cyclic ester structure at the P-O aryl bond. When the size of the exocyclic substituent R in (III) increases, to give (IV) the ester becomes a more selective inhibitor of aliesterase[88]. Whereas, it becomes a more selective inhibitor of cholinesterase when the substituent is small. Thus the O-butyl derivative, for example, is 130 times more selective to inhibit aliesterase than cholinesterase.

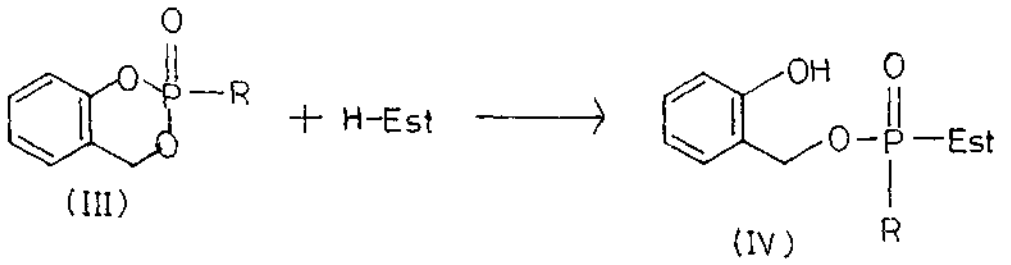


Fig. 1.7 Reaction of Saligenin cyclic phosphates with esterase (H-Est).

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

The heterocyclic structure of saligenin cyclic phosphorus esters is important merely for the chemical reactivity of the phosphorus atom towards nucleophiles including the active site of esterase and is never requirement for the delayed neurotoxicity. As for example, although Tri-o-ethyl phenyl phosphate (TEPP) has the neurotoxicity[18], is unable to be transformed into a cyclic ester structure.

Johnson found "neurotoxic esterase" in nervous tissues which is specifically sensitive *in vivo* to neurotoxic organophosphorus esters[39]. The esterase is unlike acetylcholinesterase but similar to chymotrypsin and trypsin in the structure activity relationship of inhibitors[40].

Although the structure-neurotoxicity relationship is too complicated to generalize, the neurotoxicity appears to be rather

closely related to the structure of the non leaving group than that of the leaving group.

INSECTICIDAL ACTIVITY (Table 1.6)

Saligenin cyclic methyl phosphate, methylphosphorothionate, N-methyl phosphoramidothionate are potent insecticides. It is important to note that the exocyclic substituent of the most active cyclic phosphorus ester in each series (OCH_3, SCH_3, $NHCH_3$ and CH_3CH_2) differs from each other in electronic characteristics, but resembles in steric property such as the distance (about 2.9 Å) between phosphorus and carbon atom in the P-X-C function, if the bond angle of divalent sulfur is near 90° rather than 109.5°. The introduction of any type of substituent at any position of the benzene ring and on the carbon atom of the hetero ring decreases the activity. Thus salithion is the most effective insecticide in the whole series of saligenin cyclic phosphorus esters. The P-O-C aryl bond of the hetero ring of saligenin cyclic phosphorus esters appear to be active enough to phosphorylate cholinesterase to kill insects without any electron withdrawing group.

FUNGICIDAL ACTIVITY

Salithion has no fungicidal activity, but some saligenin cyclic phosphorothioiates having S-benzyl ester linkage, have activity to protect the rice plant from rice blast desease (Pyri-

sulcata oryzae)[41]. The methyl, ethyl and n-butyl-phosphorothiolates have high fungitoxicity comparable to other commercial fungicides. The normal and isopropyl derivatives are less effective. Saligenin cyclic methyl phosphate and phosphorothionate (salithion) are highly active as insecticide but are almost inactive as fungicide.

ANTI-SH ENZYME ACTIVITY

The saligenin cyclic phosphorothiolates have high activity to alkylate (salicylate) mercaptans and to inhibit "SH-enzymes" such as yeast alcohol dehydrogenase[28]. The activity seems to be related to fungicidal property but not with the insecticidal activity.

I_{50} values for alcohol dehydrogenase of some saligenin cyclic phosphorus ester are shown in table 1.7. Cyclic methyl and ethyl phosphorothiolates are most active in this series. On the other hand, cyclic phosphates have only weak activities, though they are potent inhibitors of esterase. Salithion i.e., methyl phosphorothionate which have high insecticidal property is almost inactive toward the enzyme.

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

hydrolysate of saligenin cyclic esters react immediately with mercaptans. The reaction mechanism is shown in Fig.1.8.

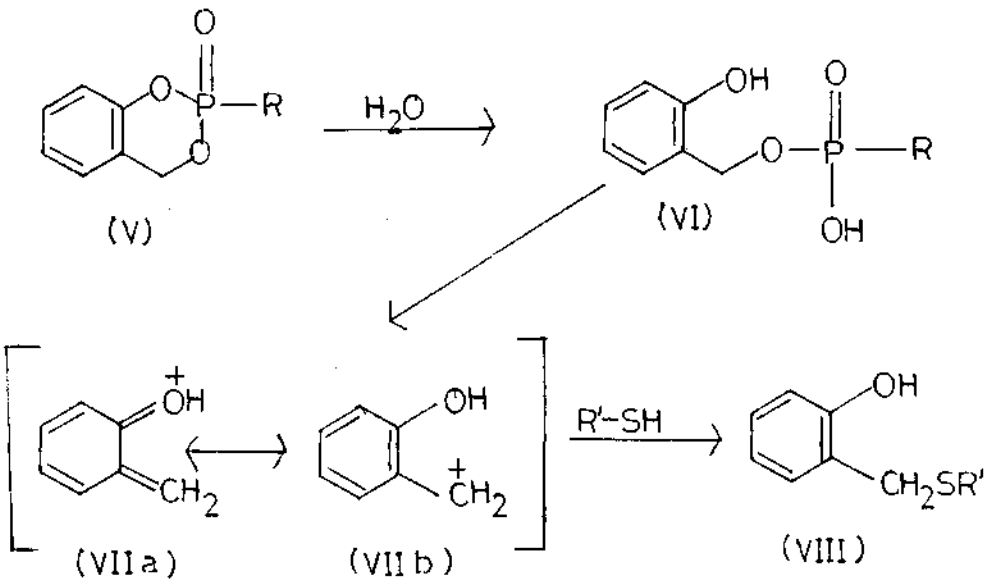


Fig. 1.8

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

a SH group to give a thioester (VIII).

Cyclic methyl and ethyl phosphorothiolates are much more active in hydrolysis, alkylation and the inhibition of "SH-enzyme" activities than the corresponding cyclic phosphates[28]. It seems reasonable to conclude that the decrease of electron density on phosphorus atom causes the high reactivity of the phosphorothiolates. This is supported by the lower P = O frequency (1260 cm^{-1}) of the phosphorothiolates in comparison with that of the phosphates (1310 cm^{-1}).

Further investigation shows(table 1.7) that there is an interesting correlation among the alkylating activity, the inhibitory activity against "SH-enzymes" and the antifungal activity of the cyclic esters. Cyclic methyl and ethyl phosphorothiolates are highly active in all three functions. Cyclic phosphates have very weak activities but they are potent inhibitors of esterases. These facts suggest that high inhibitory activity against "SH-enzymes" may be an important factor for the fungicidal activity of the cyclic phosphorothiolates.

ANTIESTERASE ACTIVITY

The most insecticidal saligenin cyclic methyl phosphate (saligen) is the strongest inhibitor of insect cholinesterase. However, the highly neurotoxic aryl phosphate is a poor inhibitor of cholinesterase, but is a very specific inhibitor of aliesterase[3,28]. The less neurotoxic arylphosphonate occupies an inter-

mediate position. In any series, when the size of the exocyclic substituent increases, the compound becomes a more selective inhibitor of aliesterase; in contrast, the compound carrying a small substituent is a more selective inhibitor of cholinesterase (Table 1.8). Aryl phosphonates are more specific inhibitors of Pseudo-cholinesterase; alkyl phosphates are less specific and aryl phosphates are intermediate.

SYNERGISTIC ACTIVITY

Saligenin cyclic aryl phosphates and phosphonates have synergistic activity with malathion against insects and mites, particularly their resistant strain[42].

The joint action of the activity of some saligenin cyclic phosphorus esters with malathion has been examined by Eto, Oshima, Kitakata, Tanaka and Kojima[37] and compared with some phosphorus esters which are known as the synergists of malathion. They increase the toxicity of malathion 2.3 to 3.4 times at a 1:1 mixing ratio. The activities of them are more than propyl paraoxon but less than Dibrom and isopropyl paraoxon.

It has been observed that for a large number of organo-phosphorus compounds the synergism of malathion in mice and the degree of inhibition of ali-esterase *in vivo* are generally related[12]. For insects, high esterase activity hydrolysing malathion is supposed to be partly responsible for malathion-

resistance in some strains of mosquito, housefly and green rice leaf hopper[37].

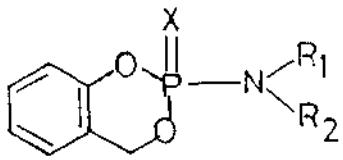
Eto et al[38] have shown that aryl derivatives of saligenin cyclic phosphorus esters are the selective inhibitors of aliesterase, whereas small alkyl derivatives are not so selective to aliesterase inhibition. This appears to be responsible for their difference in synergistic properties.

Conclusion

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

Table 1.6

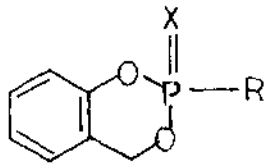
Saligenin cyclic phosphoramidates and phosphoramidothionates: insecticidal activity and toxicity.



X	$-N\begin{matrix}R_1\\R_2\end{matrix}$	LD_{50} (μg/female House fly	LD_{50} (mg/kg (mouse)
O	NHMe	0.05	5-7.5
O	NHEt	0.60	30-50
O	NH-n-Pr	1.20	>50
O	NH-n-Bu	<10(54%)	>50
O	NH-Ph	>10 (5%)	-
O	$N(Me)_2$	0.3	-
O	$N(Et)_2$	>10 (0%)	>50
S	NHMe	0.044	20-30
S	NHEt	0.48	-
S	$N(Me)_2$	0.28	-
S	$N(Et)_2$	0.63	-
S	OMe	0.05	85
O	OMe	0.035	32
Parathion		0.040	3-7
Malathion		0.060	347

Table 1.7

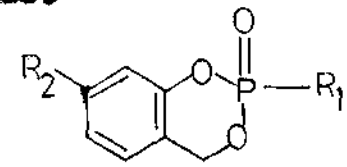
Chemical, Biological and Anti-Fungal activities of some Saligenin cyclic phosphates and their thio analogues.



X	R	Hydrolysis(%)	Cysteine reacted (%)	I_{50} Yeast alcohol dehydrogenase ($\mu \times 10^{-5}$)	Protective value against *Piricularia Oryzae* (%) 50 ppm	500 ppm
O	SCH_3	86	55	4.5	100	-
O	SC_2H_5	81	50	4.4	93	-
O	OC_6H_5	55	40	6.8	-	-
O	OCH_3	17	10	62	-	65
S	OCH_3	9	5	100	-	52

Table 1.8

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



R_1	R_2	Housefly $I_{50} \times 10^8 (M)$		Human blood $I_{50} \times 10^8 (M)$		Horse plasma $I_{50} \times 10^8 (M)$	
		ChE	AlkE	P-ChE	B-ChE	AlkE	Malathionase
OCH_3	H (Saligenin)	7.6	8.4	1.8	17.0	280	620
OC_2H_5	H	13.2	2.1	1.6	25.0	240	-
O-n-C_3H_7	H	30.7	3.0	-	-	-	-
O-n-C_4H_9	H	37.5	2.3	-	-	-	-
OC_6H_5	H	-	-	0.5	12.0	120	120
C_6H_5	H	-	-	0.65	72.0	180	470
C_6H_5	CH_3	-	-	1.6	68.0	230	-
OPh-2-CH_3	H	-	-	1.3	39.0	200	-

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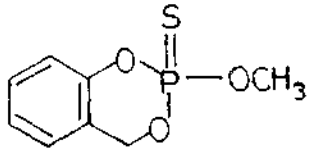
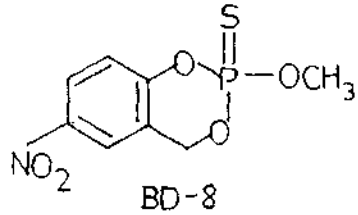
1.3 AIMS AND OBJECTIVES OF THE PRESENT INVESTIGATION

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



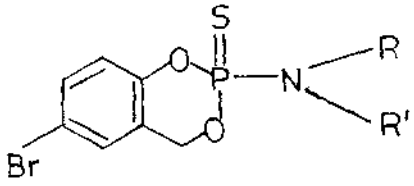
Salithion



BD-8

However, it has been observed by Das[4] that the said methoxy compound (BD-8) is a solid (m.p. 84°C), and has about 1.5-2 times greater oral insecticidal activity to cockroach, *Periplaneta americana* (Linn) compared with salithion. These observations prompted us to undertake a systematic investigation on some substituted saligenin cyclic phosphorus compounds. This work is mainly concerned with the chemical, spectral, biochemical, chemical hydrolysis, insecticidal, phytotoxic and fungicidal

properties of some 2-alkylamido-6-bromo-4H-1,3,2-benzodioxaphosphorin 2-sulphides of the general structure given below.



where $-N\begin{matrix}R\\R^1\end{matrix}$ = Diisobutylamido

= Dipentylamido

= Dihexylamido

= Dibutylamido

= 2,6-Dimethylmorpholino

= 2-Ethylpiperidino

= 4-Benzylpiperidino

= Hexamethyleneimido

ACTUAL WORK

1. Synthesis :

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

2. Spectral Studies :

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

3. Studies of Biological Properties :

(i) Insecticidal activity test of these compounds on cockroach has been performed.

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

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

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

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

4. Studies of Chemical Hydrolysis :

Chemical hydrolysis of these compounds at alkaline pH 11.50 has been given.

5. Quantitative Structural Activity Relationship :

Relationship between chemical structure and antifungal activities has been studied using different parameters, such as hydrophobic constant (log P or π), Taft's steric constant (E_s) and Structural Information Content (SIC) of the various molecules.

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CHAPTER 3

SYNTHESIS AND SPECTRAL PROPERTIES OF BIS(2-ALKYLAMIDO-4-PHENO)BALLAGENIA CETRIC INDOPHENOLDITHIONATES

Chapter - 2

SYNTHESIS AND SPECTRAL PROPERTIES OF SOME 2-ALKYLAMIDO 4-BROMO SALIGENIN CYCLIC PHOSPHORAMIDOTHIONATES

MATERIALS AND METHODS

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

2.1 PURIFICATION OF SOLVENTS AND CHEMICALS

Organic solvents and chemicals used during the work were of standard commercial grade of high quality (EM, BDH, Sd, 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.

2.2 SPECTROSCOPIC METHODS

Infrared spectra were scanned on Beckmann IR-20 spectrophotometer and Pye Unicam Sp 3 3006 in nujol mull and in liquid film. Ultraviolet - spectra were recorded on Shimadzu UV-240 u.V. Visible spectrophotometer (Graphicord) in ethanol. Mass spectra (EI positive) were recorded on a Jeol JMS - D300 mass spectrometer at 70 eV. NMR spectra were taken on varian EM 390-L, Varian CFT-20 and Jeol spectrometers. ^{13}C spectra were recorded on Bruker WM-400 spectrometer at 100 MHz. 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.

2.3 PREPARATION OF THIOPHOSPHORYL CHLORIDE ($PSCl_3$)

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

2.4 PREPARATION OF 5-BROMO SALIGENIN (2-HYDROXY-5-BROMOBENZYL ALCOHOL)

2-hydroxy 5 bromo benzyl alcohol for the synthesis of 6-bromo-saligenin cyclic phosphoramidothionate was prepared in the following manner :

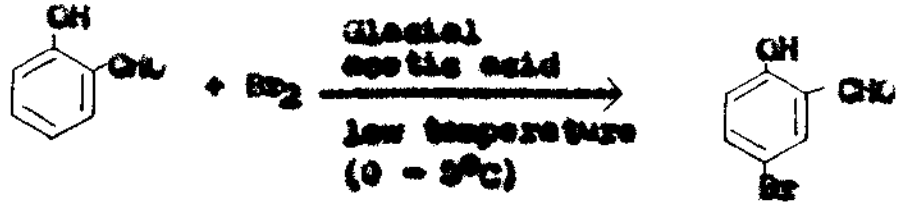
Alcohol was prepared in two stages -

(i) bromination of salicylaldehyde to 2-hydroxy-5-bromo benzaldehyde, and

(ii) reduction of the said aldehyde to alcohol.

(i) Bromination of Salicylaldehyde

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

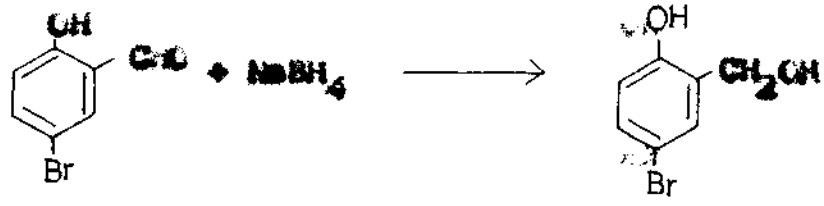


12.2 g (0.1 mole) Salicylaldehyde and 17 g (0.1 mole) liquid bromine were dissolved in 40 ml and 25 ml glacial acetic acid

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 3°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 into 400 ml cold water and more crystals 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 16.1 g (80% yield).

(ii) <u>Reduction of 2-hydroxy-5-bromobenzaldehyde</u>

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 solution of 20.1 g (0.1 mole) 2-hydroxy-5-bromobenzaldehyde in 100 ml methanol and, whilst stirring, a solution of sodium borohydride (1.4 g, 0.037 mole sodium borohydride in 2 ml of 2N-sodium hydroxide diluted with 18 ml of water) was added at a rate of

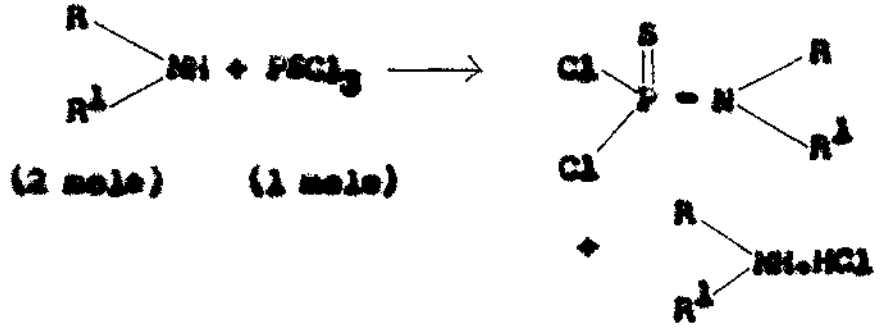
0.5 ml per minute with occasional cooling to keep the reaction mixture at 18° - 23°C. The reaction mixture was stirred for an additional period of 30 minutes.

The methanol was removed by distillation on a steam bath. The residue was diluted with 100 ml water and then acidified with 30 ml dilute sulphuric acid. The mixture was extracted with ether, the upper layer of the extract was washed with water to remove excess acid and dried with a little anhydrous magnesium sulphate. Ether was removed by flash distillation. The product thus obtained was recrystallized from hot chloroform, and dried in vacuum. The yield of pure 2-hydroxy-5-bromobenzyl alcohol, (colourless crystals, m.p. 108°C) was 17.1 g (85% yield).

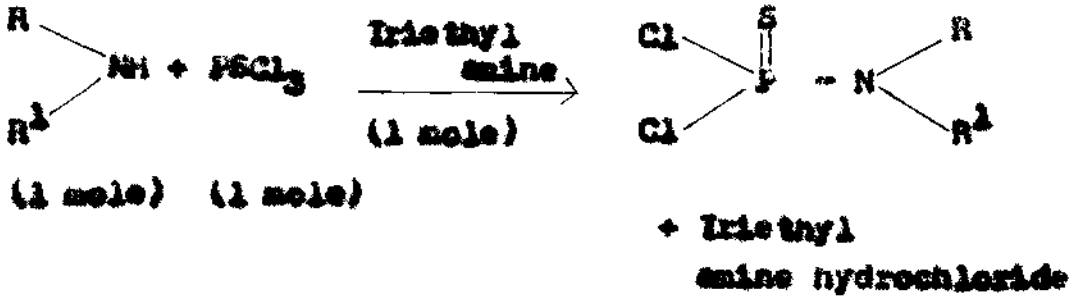
uv (Fig.2.1): λ_{max}^{EtOH}, 284 nm (ϵ, 2080).

IR (Fig.2.2): 3420 and 3140 cm^{-1}, OH vibration; 1480 cm^{-1}, OH deformation str. vibration; 1610 and 1590 cm^{-1}, C=C str.; 1450 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}, the lone H-atom wagging of phenyl ring; 810 cm^{-1}, bending of 2H adjacent of the ring.

2.5 PREPARATION OF ALKYLAMIDOPHOSPHOROCHLORIDOTHIONATES



or,



One mole $PSCl_3$ and two moles amine (or one mole amine and one mole triethylamine) were allowed to react at -8°C to +5°C in benzene/chloroform as solvent. The amine solution was added dropwise very slowly with constant vigorous stirring. After an additional stirring period, the solid particles (if present) were filtered off the reaction mixture and washed repeated with benzene/chloroform. Excess amine was removed by washing the benzene/chloroform phase with 2% cold hydrochloric acid, then with cold saturated solution of sodium chloride. The benzene/chloroform phase was then dried with anhydrous sodium sulphate and filtered, evaporation *in vacuo* gave the desired alkylamidophosphorodichloridothionate.

The different alkylamidophosphorodichloridothionates were prepared as follows :

2.5.1 N-N-Di isobutylamidophosphorodichloridothionate :

A solution of diisobutylamine (12.9 g; 0.1 mole) in 20 ml benzene was added dropwise to a stirred solution of thiophosphoryl chloride (8.45 g; 0.05 mole) in 50 ml benzene at -5° to + 5°C. The mixture was stirred at 5°C for 3 hrs and then at room temperature for 16 hrs. 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 10 g N-N-diisobutylamidophosphorodichloridothionate. This compound has a camphor - like odour.

2.5.2 N-N-Dipentylamidophosphorodichloridothionate :

Thiophosphoryl chloride (8.45 g, 0.05 mole) in 50 ml benzene was allowed to react with 19.7 g (0.1 mole) dipentylamine in 20 ml benzene, and the mixture was worked up as in 2.5.1 . 12.5 g liquid was obtained. This compound has a camphor-like odour.

2.5.3 N-N-Dibenzylamidophosphorodichloridothionate :

Thiophosphoryl chloride (8.45 g; 0.05 mole) in 50 ml benzene was allowed to react with 19.5 g (0.1 mole) of dibenzylamine in 20 ml benzene, and the mixture was worked up as in 2.5.1. 14.3 g liquid

was obtained. This compound has a camphor-like odour.

2.3.4 <u>N-N-Dibutylamidophosphorodichloridothionate</u> :

Thiophosphorylchloride (6.45 g, 0.05 mole) in 50 ml benzene was allowed to react with 6.5 g (0.05 mole) of dibutylamine and 5 g (0.05 mole) of triethylamine in 20 ml benzene, and the mixture was worked up as in 2.3.1. 5g liquid was obtained. This compound has a camphor-like odour.

2.3.5 <u>2,6-Dimethylmorpholinophosphorodichloridothionate</u> :

Thiophosphoryl chloride (16.90 g; 0.1 mole) in 50 ml benzene was allowed to react with 11.5 g (0.1 mole) of 2,6-dimethylmorpholine and 10.1 g (0.1 mole) of triethylamine in 20 ml benzene, and the mixture was worked up as in 2.3.1. 10.2 g liquid was obtained. This compound has a camphor-like odour.

2.3.6 <u>2-Ethylpiperidinophosphorodichloridothionate</u> :

16.90 g (0.1 mole) thiophosphoryl chloride in 50 ml benzene was allowed to react with 11.3 g (0.1 mole) of 2-ethylpiperidine and 10.1 g (0.1 mole) of triethylamine in 20 ml benzene, and the mixture was worked up as in 2.3.1. 10 g liquid was obtained. This compound has a camphor-like odour.

2.3.7 <u>4-Benzylpiperidinophosphorodichloridothionate</u> :

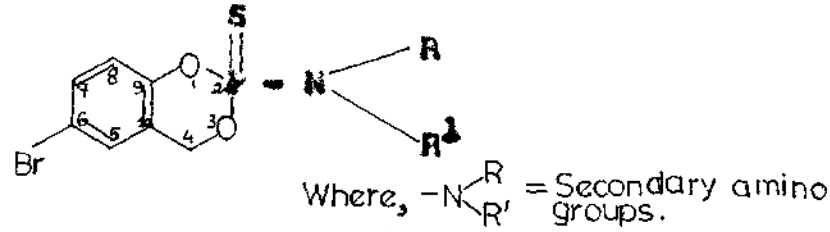
Thiophosphoryl chloride (8.45 g, 0.05 mole) in 50 ml benzene was allowed to react with 8.8 g (0.05 mole) of 4-benzyl piperidine

and 5 g (0.05 mole) of triethylamine in 20 ml benzene, and the mixture was worked up as in 2.5.1. 6 g liquid was obtained. This compound has a camphor-like odour.

2.5.3 <u>Hexamethylenimidophosphorodichloridothionate</u> :

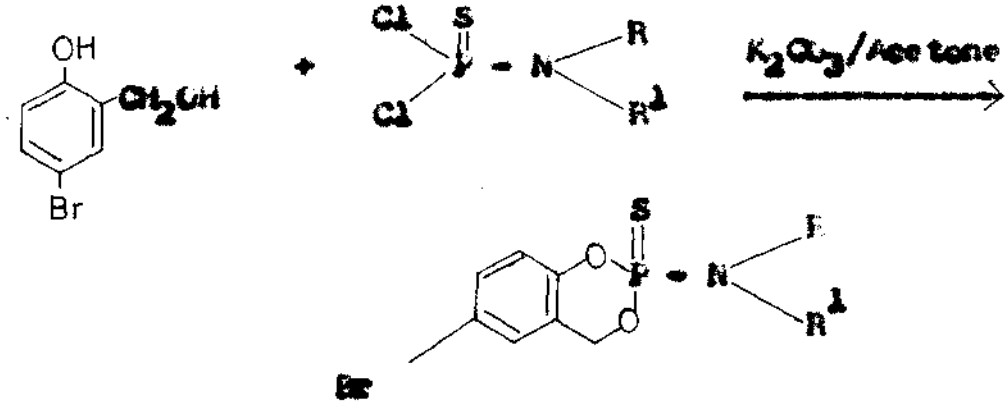
Thiophosphoryltrichloride (16.90 g; 0.1 mole) in 50 ml benzene was allowed to react with 9.9 g (0.1 mole) of hexamethylenimine and 10.1 g (0.1 mole) of triethylamine in 20 ml benzene, and the mixture was worked up as in 2.5.1. 10 g liquid was obtained. This compound has also a camphor-like odour.

2.6 <u>PREPARATION OF SOME 2-ALKYLAMIDO-6-BROMO-4H-1,3,2-BENZODIOXAPHOSPHORIN 2-SULPHIDES</u>

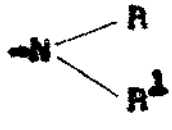


In general 2-alkylamido-6-bromo-4H-1,3,2-benzodioxaphosphorin 2-sulphides were prepared by adding a solution of 2-hydroxy-5 bromobenzylalcohol (5-bromo saligenin, 1 mole) in dry acetone to 1 mole of alkylamidophosphorodichloridothionate with cooling in an ice-bath. Dry anhydrous potassium carbonate (2 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 accom-

plished by stirring at the temperature 5-27°C for an additional time of 12-16 hrs. The solid particles were filtered out of the reaction mixture and the solvent was removed under reduced pressure at room temperature. In some cases the crude product was directly 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 annydrous sodium sulphate and the chloroform was subsequently removed under reduced pressure. The pure compound was then obtained by recrystallization from methanol.



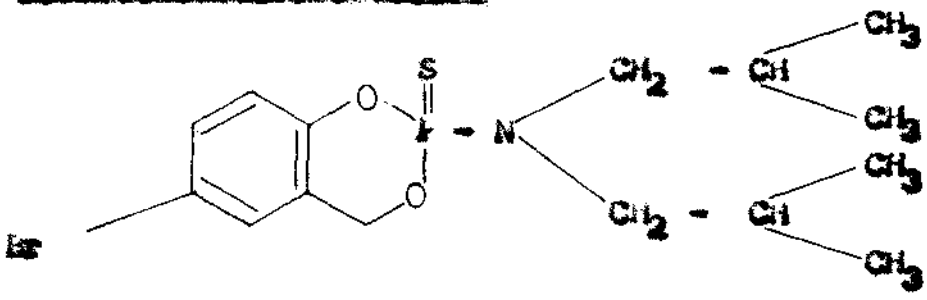
where,



= Secondary amine groups

The different phosphoramidothionates were prepared as follows :

2.6.1 <u>2-N,N-Diisobutylamido-6-bromo-4H-1,3,2-benzodioxaphosphorin 2-sulfide (BH-6)</u>



This compound (BH-6) was prepared by condensation of 2-hydroxy-5-bromobenzylalcohol (2.03 g, 0.01 mole) and N,N-diisobutylamidophosphorodichloridothionate (2.42 g, 0.01 mole) in presence of K_2CO_3 (2.76 g, 0.02 mole) in 30 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 recrystallized from hot methanol. 3.32 g compound (BH-6) was obtained as a white crystalline solid.

Yield, 85%; Mol. Formula: $C_{15}H_{23}O_2NPSBr$; Mol.wt., 391.29; M.P., 99°; R_f, 0.91 (benzene:acetone, 8:2).

Anal. Calcd for: C,46.01 ; H, 5.92; N, 3.58. Found for : C,46.18; H, 5.89; N, 3.59.

UV(Fig.23): λ_{max}^{EtOH}, 276 nm (ε ,1600).

IR(Fig.2.4): 1013 cm^{-1}(S), r-C-C alkyl: 1235-1260 cm^{-1}(S) and 940 cm^{-1}(S), r-C-C aryl: 610 cm^{-1}(S), r = S(1): 650 cm^{-1}, r = S(II): 1040 cm^{-1}(S), Ar-Br: 730 cm^{-1}(S), r-N str.

Mass(Fig.2.5): $\frac{M}{Z}$ 393$(M+2)^+$ 391(M^+)

		MRI	19		15
358	348	292	263	231	174
20	Basepeak	65	20	22.5	15
39	43				
62.5	40				

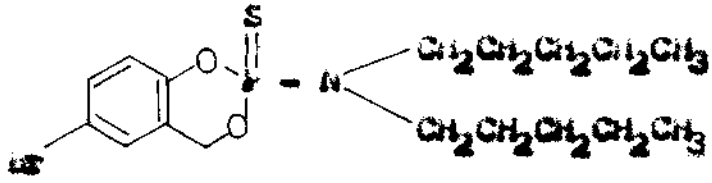
^1H NMR (Acetone-d_6/TMS)ppm (Fig.2.6): δ 0.70 - 0.95 (d,12H, $-CH_3$gr.), 1.20 - 2.30 (m, 2H - CH< gr.), 2.80 - 3.20 (m, 4H, $-N(CH_2)_2$ gr.), 4.90 - 5.60(eight line m, 2H, $-CH_2-(-I)$, 6.80 - 7.45 (m, 3H, aromatic).

^{13}C NMR ($CDCl_3$/TMS)ppm (Fig. 2.7):

^{13}C Atom	Values (ppm)	$^{n}J^{*}_{AB}$	Coupling constant (Magnitude in Hz).
C_3'	19.92 19.80	$^{4}J_{P-N-C_1'-C_2'-C_3'}$	11.68
C_2'	25.64		
C_1'	32.54		
C_4	66.24		
C_5	115.65		
C_8	120.63 120.55	$^{3}J_{P-C-C_9-C_8}$	6.78
C_{10}	122.67 122.56	$^{3}J_{P-C-C_9-C_{10}}$	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 & B.

2.6.2 2-(N,N-dipentylamido)-6-bromo-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BR-8)



This compound (BR-8) was prepared by condensation of 2-hydroxy-5-bromobenzyl alcohol (2.03 g, 0.01 mole) and N, N-dipentylamidophosphorodichloridothionate (2.9 g, 0.01 mole) in presence of K_2CO_3 (2.76 g, 0.02 mole) in 50 ml acetone as solvent; K_2CO_3 was added by instalments to the stirred solution at 0° to 5°C. After 18h stirring the solid was filtered off and the solvent (acetone) was removed under reduced pressure at room temperature. 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 *in vacuo* 3.14 g of product BR-8 (liquid) was obtained.

Yield, 75%; Mol. Formula, $C_{17}H_{27}O_2N_2SBr$; Mol. wt., 419.33; R_f, 0.84 (Benzene:acetone, 9:1). Anal. Calcd for: C, 48.65; H, 6.44; N, 3.34.

Found for : C, 48.73; H, 6.44; N, 3.35.

UV(Fig.28): λ_{max}^{EtOH}, 278 nm (ϵ,1334).

IR(Fig.29): 1040 cm^{-1}(S), r-O-C alkyl; 1250-1260 cm^{-1}(S) and 890 cm^{-1}(S), r-O-C aryl; 810 cm^{-1}(S), r = S(1); 690 cm^{-1}(S), r = S(II); 1460 cm^{-1}(S), Ar-Br; 730 cm^{-1}(S), r-N str.

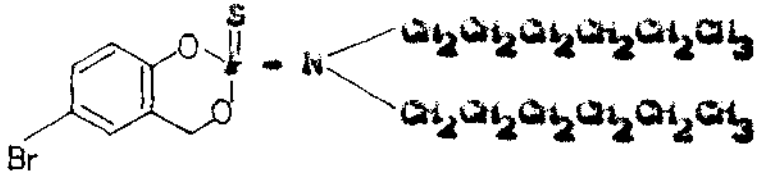
mass (Fig.210): $\frac{m}{z}$ 421(M^{+2})$^+$ 419(M^+) 386 362

%Ra 10 10 72.5 16.25

305	292	202	184	156	43
22.5	11.25	20	67.5	42.5	basepeak

^{1}H NMR ($CDCl_3$/TMS)ppm (Fig.211): δ 0.84-1.32(m, 18H, $-(CH_2)_3CH_3$ gr.), 2.16-3.40 (m, 4H, $-N(CH_2)_2$ gr.), 4.79-5.74 (eight line m, 2H, $-CH_2-O-P$), 7.10-7.31 (m, 3H, aromatic).

2.6.3 2-thi - dihexylamido-6-bromo-4r-1,3,2-benzodioxaphosphorin 2-sulphide (BH-9)



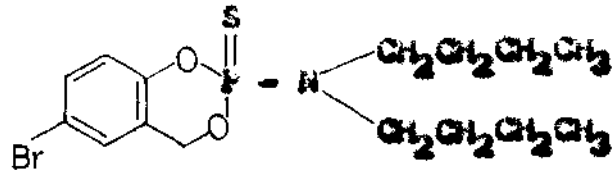
The condensation of 2-hydroxy-3-bromobenzyl alcohol (2.03 g, 0.01 mole) and N,N-dihexylamidophosphorodichloridothionate (3.18 g, 0.01 mole) in presence of K_2CO_3(2.76 g, 0.02 mole) in 50 ml acetone gave BH-9 as liquid. BH-9 was purified as in 2.6.2.

After removal of solvent in vacuo 3.36 g of colourless liquid product was obtained.

Yield, 75%; Mol. Formula, $C_{19}H_{31}O_2NPSBr$; Mol.wt., 447.36;
R_f, 0.86 (benzene:acetone, 9:1).
Anal. calcd for : C, 50.96; H, 6.98; N, 3.13.
Found for: C, 50.90; H, 6.90; N, 3.11.
UV (Fig.2.12): λ_{max}^{EtOH}, 279 nm (ϵ,1332).
IR (Fig.2.13): 1020 cm^{-1}(S), r-O-C alkyl; 1240-1260 cm^{-1}(S) and 900 cm^{-1}(S), r-O-C aryl; 805 cm^{-1}(S), r-S(I); 650 cm^{-1}, r-S(II), 1060 cm^{-1}(S), Ar-Br; 730 cm^{-1}(S), r-N str.

^1H NMR (Acetone-d_6/TMS)ppm (Fig.2.14):
δ 0.65-1.50 (m, 22H, $-(CH_2)_4CH_3$ gr.), 2.90-3.40 (m, 4H, $-N(CH_2)_2$ gr.), 4.65-5.60 (m, 2H, $-CH_2-O-P$), 6.50-7.45 (m, 3H, aromatic).

2.6.4. <u>2-N,N-Dibutylamido-6-bromo-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BF-10)</u>



The condensation of 2-hydroxy-5-bromobenzyl alcohol (2.03 g, 0.01 mole) and N,N-dibutylamidophosphorodichloridothionate (2.62 g,

0.01 mole) in presence of K_2CO_3 (2.76 g, 0.02 mole) in 50 ml acetone gave BR-10 as liquid. BR-10 was purified as in 2.6.2. After removal of solvent *in vacuo* 2.70 g of colourless liquid product was obtained.

Yield, 70%; Mol. Formula, $C_{19}H_{23}O_2N_2SBr$; Mol.wt., 391.25; R_f, 0.85 (benzene:acetone, 9:1). Anal. Calcd for: C, 46.00; H, 5.85; N, 3.38. Found for: C, 46.21; H, 5.90; N, 3.60.
UV (Fig.2.15): λ_{max}^{EtOH}, 278 nm (ϵ,1162).

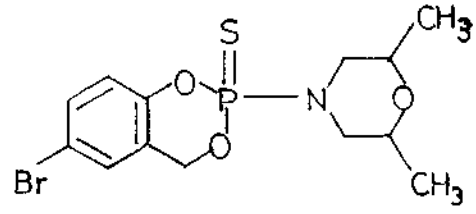
IR (Fig.2.16): 1320 cm^{-1}(S), C-O-C alkyl: 1250-1260 cm^{-1}(S) and 930 cm^{-1}(S), C-O-C aryl: 810 cm^{-1}(S), C=S(I): 650 cm^{-1}(S), C=S(II): 1050 cm^{-1}(S), Ar-Br: 730 cm^{-1}(S), C-N str.

Mass (Fig.2.17) $\frac{m}{z}$ 393($M+2$)$^+$ 391(M^+)

		%RI	65		37.5
358	348	305	292	263	231
Basepeak	22.5	22.5	16.25	10	23.75
174	57				
50	17.5				

^{1}H NMR (Acetone-d_6/TMS)ppm (Fig.2.18):
δ 0.80-0.90 (t, 6H, $-CH_3$ gr.), 1.20-2.10 (m, 8H, $-CH_2-$gr.), 2.91-3.40 (m,4H, $-N(CH_2)_2$ gr.), 4.73-5.56 (m, 2H, $-CH_2-O-P$), 6.80-7.46 (m, 3H, aromatic).

2.6.5. 2-(2,6-Dimethylmorpholino)-6-bromo-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BR-12)



The condensation of 2-hydroxy-5-bromobenzyl alcohol (2.03 g, 0.01 mole) and 2,6-dimethylmorpholinophosphorodichloridothionate (3.78 g, 0.01 mole) in presence of K_2CO_3 (2.76 g, 0.02 mole) in 50 ml acetone gave BR-12. Recrystallization from methanol gave 3 g of product as white crystalline solid.

Yield, 82%; Mol. Formula, $C_{13}H_{17}O_3NSBr$; Mol. wt., 377.18; M.P., 75°; R_f 0.84 (benzene:acetone, 8:2).

Anal. Calcd for : C, 41.36; H, 4.50; N, 3.71.

Found for : C, 41.38; H, 4.49; N, 3.73.

UV (Fig.2.19): λ_{max}^{EtOH}, 278 nm (ϵ 9943).

IR (Fig.2.20): 1020 cm^{-1}(S), P-O-C alkyl; 1250-1260 cm^{-1}(S) and 870 cm^{-1}(S), P-O-C aryl; 805 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.2.21): m/z 379(M^++2)$^+$ 377(M^+) 362

XRI 22.61 22.61 Base-peak

344 263 231 160

11.9 14.28 27.38 8.33

^{1}H NMR (Acetone-d_6 & $CDCl_3$/DMS)ppm (Fig.2.22) :

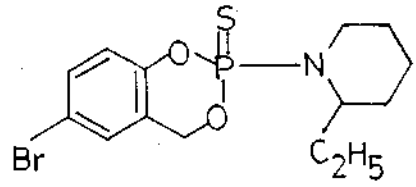
δ 1.11 - 1.60 (d, 6H, $-CH_3$gr.), 2.12 - 2.80 (m, 4H, $-N(CH_2)_2$gr.), 3.31 - 3.80 (m, 2H, CH-C-CH), 4.87 - 5.73 (eight line m, 2H, $-CH_2$-C-H), 6.81 - 7.43 (m, 3H, aromatic).

^{13}C NMR ($CDCl_3$/DMS) ppm (Fig.2.23):

^{13}C Atom	Values (ppm)	$^{n}J^{*}_{AB}$	Coupling constant (magnitude in Hz).
$C_{3'}$	18.48		
$C_{1'}$	50.20		
$C_{2'}$	71.93		
C_4	66.23		
C_5	115.99		
C_8	120.63 120.55	$^{3}J_{1-O-C_9-C_8}$	8.32
C_{10}	122.30 122.39	$^{3}J_{1-O-C_9-C_{10}}$	10.32
C_7	127.91		
C_6	132.13		
C_9	149.91 149.86	$^{2}J_{1-O-C_9}$	5.78

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

2.6.6. 2-(2-Ethylpiperidino)-6-bromo-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BF-14)



The condensation of 2-hydroxy-5-bromobenzylalcohol (2.03 g, 0.01 mole) and 2-ethylpiperidinophosphorodichloridothionate (2.46 g, 0.01 mole) in presence of K_2CO_3 (2.76 g, 0.02 mole) in 50 ml acetone gave BF-14. Recrystallization from methanol gave 3.4 g of product as white crystalline solid.

Yield, 90%; Mol. Formula, $C_{14}H_{19}O_2NPSBr$; Mol. wt., 375.20; M.P., 105°; Rf, 0.81 (benzene:acetone, 6:2).

Anal. Calcd for : C, 44.77; H, 5.06; N, 3.73.

Found for : C, 44.98; H, 5.10; N, 3.75.

UV (Fig.2.24) : λ_{max}^{EtOH}, 278 nm (ϵ ,1365).

IR (Fig.2.25) : 1010 cm^{-1}(S), P-O-C alkyl; 1240-1260 cm^{-1}(S) and 880 cm^{-1}(S), P-O-C aryl; 810 cm^{-1}(S), P=S(I); 650 cm^{-1}(S), P=S(II); 1300 cm^{-1}(S), Ar-Br; 730 cm^{-1}(S), P-N str.

Mass (Fig.2.26) :

		m/z	377(M+2)$^+$	375(M$^+$)
		%RI	30.95	30.95
346	342	231	158	
Basepeak 90.47		8.33	47.62	

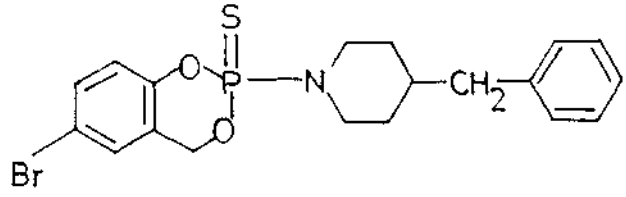
^{1}H NMR ($CDCl_3$/TMS)ppm (Fig.2.27) : δ 0.85-1.05 (t, 3H, $-CH_3$gr. in the side chain of piperidine ring), 1.50-1.62 (d, 8H, $-CH_2-$gr.), 3.05-3.50 (m,1H, $-N<_{CH-}$ gr.), 3.50-4.05 (m, 2H, $-N<^{CH_2-}$ gr.), 4.80-5.70 (eight line m, 2H, $-CH_2-O-P$), 6.80-7.40 (m,3H, aromatic).

^{13}C NMR ($CDCl_3$/TMS) ppm (Fig. 2.28):

^{13}C Atom	Values (ppm)	$^{n}J^{*}_{AB}$	Coupling constant (magnitude in Hz).
C_7'	19.81		
C_6'	18.80		
C_3'	22.54		
C_4'	23.94		
C_2'	28.38		
C_5'	40.03		
C_1'	53.94		
C_4	66.24		
C_9	115.54		
C_8	120.37 120.35	$^{3}J_{H-O-C_9-C_8}$	8.25
C_{10}	122.67 122.35	$^{3}J_{H-O-C_9-C_{10}}$	11.03
C_7	127.82		
C_6	131.84		
C_9	150.33 150.29	$^{2}J_{H-O-C_9}$	6.00

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

2.6.7. 2-(4-benzylpiperidino)-4-bromo-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BR-15).



To a stirred solution of 2.03 g (0.01 mole) of 2-hydroxy 5-bromobenzyl alcohol and 3.38 g (0.01 mole) of 4-benzylpiperidinophosphorodichloridothionate was added 2.76 g (0.02 mole) of anhydrous potassium carbonate at 0° to 5°. After 18h the solid was filtered off and the solvent (acetone) was removed under reduced pressure. The combined filtrate was washed with methanol saturated with n-heptane and the residue was purified by column chromatography (silica gel) using benzene-chloroform (9:1) as eluent. On evaporation of solvent a 3.50 g colourless high viscous liquid product BR-15 was obtained.

Yield, 80%; Mol. Formula, $C_{19}H_{21}Cl_2NO_2Br$; Mol. wt., 437.28;
R_f, 0.92 (benzene:acetone, 8:2).
Anal. Calcd for : C, 52.14; H, 4.80; N, 3.20
Found for : C, 52.31; H, 4.82; N, 3.25
UV (Fig.2.29) : λ_{max}^{EtOH}, 273 nm (ϵ,1404).
IR (Fig.2.30) : 1020 cm^{-1}(S), P-O-C alkyl; 1240-1260 cm^{-1}(S) and 900 cm^{-1}(S), P-O-C aryl; 830 cm^{-1}(S), P=S(I); 640 cm^{-1}(S), P=S(II); 1050 cm^{-1}(S), Ar-Br; 725 cm^{-1}(S), P-N str.

Mass (Fig.2.31): $\frac{m}{z}$ 439 $(M+2)^+$ 437 (M^+)

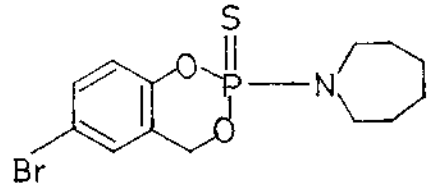
XRI 11.90 11.31

404	231	220	174	91
80.95	9.52	21.42	16.66	Basepeak

^1H NMR ($CDCl_3$/DMS)ppm (Fig.2.32):

δ 1.04-1.75 (m,3H, $-CH(CH_2)_2$gr.), 2.65-3.30 (m, 4H, $-N(CH_2)_2$gr.), 3.40-3.93(m, 2H, $-CH_2-$gr.), 4.60-5.74 (triplet like m, 2H,$-CH_2-O-P$), 6.84-7.14 (m, 8H, aromatic).

2.6.8. <u>2-Hexamethyleneimido-6-bromo-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BR-17)</u>



The condensation of 2-hydroxy 5-bromobenzyl alcohol (2.03g, 0.01 mole) and hexamethyleneimidophosphorodichloridothionate (2.32 g, 0.01 mole) in presence of K_2CO_3 (2.76 g, 0.02 mole) in 50 ml acetone gave BR-17. Recrystallization from methanol gave 2.5 g of product as white crystalline solid.

Yield, 73%; Mol. Formula, $C_{12}H_{17}O_2NPSBr$; Mol. wt., 361.18; M.P., 101°, R_f, 0.89 (benzene : acetone, 8:2).

Anal. Calcd for : C, 39.87; H, 4.71; N, 3.88. Found for : C, 40.14, H, 4.90, N, 4.00.

UV(Fig.2.33) : λ_{max}^{EtOH}, 278 nm (ϵ,1347).

IR(Fig.2.34) : 1010 cm^{-1}(S), P-O-C alkyl; 1240-1260 cm^{-1}(S) and 870 cm^{-1}(S), P-O-C aryl; 815 cm^{-1}(S), P=S(I); 640 cm^{-1}(S), P=S(II); 1065 cm^{-1}(S), Ar-Br; 730 cm^{-1}(S), P-N str.

Mass(Fig.2.35) : $\frac{m}{z}$ 363 $(M+2)^+$ 361(M^+)

%RI 37.14 54.76

328 231 144 98

Basepeak 7.14 37.14 16.66

^1H NMR ($CDCl_3$/TMS)ppm (Fig.2.36) :

δ 1.50-1.70 (m, 8H, $-CH_2-$gr.), 3.26-3.41 (m, 4H, $-N(CH_2)_2$gr.), 4.80-5.70 (m, 2H, $-CH_2-O-P$), 6.80-7.40 (m, 3H, aromatic).

^{13}C NMR ($CDCl_3$/TMS)ppm (Fig.2.37):

^{13}C Atom	Values (ppm)	$^{n}J^{*}_{AB}$	Coupling constant (Magnitude in Hz)
C_3'	28.39		
C_2'	29.81		
C_1'	43.47		
C_4	64.04		
C_5	115.67		
C_8	120.33 120.48	$^{3}J_{P-O-C_9-C_8}$	7.07
C_{10}	122.76		
C_7	127.89		
C_6	131.96		
C_9	150.16		

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

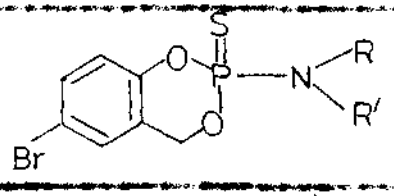
2.7 RESULTS AND DISCUSSION:

2.7.1 DISCUSSION ON SYNTHESIS :

Some bromo saligenin cyclic phosphoramidothionates such as BR-6, BR-8, BR-9, BR-10, BR-12, BR-14, BR-15 and BR-17 have been synthesized. Of them compound BR-6, BR-12, BR-14 and BR-17 are solid and have been recrystallized from methanol. The compound BR-8, BR-9, BR-10 and BR-15 are colourless liquid and have been purified by column Chromatography in pure form. All these compounds have been prepared in good yields. The percent yield and melting point of these compounds are given in table 2.1.

Table 2.1

Percent yield and melting point of different 2-alkylamido-6-bromo saligenin cyclic phosphoramidothionates.



Alkylamido group	Compound code No.	Yield(%)	M.P. (°C)
Diisobutylamido	BR-6	90	90
Dipentylamido	BR-8	75	liquid
Dimethylamido	BR-9	75	liquid
Dibutylamido	BR-10	70	liquid
2-6-Dimethyl-morpholino	BR-12	82	75
2-Ethylpiperidino	BR-14	90	105
4-Benzylpiperidino	BR-15	80	liquid
Hexamethyleneimido	BR-17	70	101

2.7.2 DISCUSSION OF IR SPECTRA

The IR spectra of the 6-bromo saligenin cyclic phosphoramidothionates have been analysed according to Thomas[3], Bellamy[4], Coltman et al[5] and Das[6]. The common IR bands for all compounds are summarized below :

1010 - 1040 cm^{-1}(S), P-O-C (alkyl);
1235 - 1260 cm^{-1}(S) and
870 - 910 cm^{-1}(S), P-O-C (aryl);
805 - 815 cm^{-1}, P = S (I);
640 - 690 cm^{-1}, P = S(II);

The thionogroup is characterized by two IR absorption bands with frequencies in the normal ranges given by Thomas[3], as both are not observed in amidophosphates; of these two, the lower frequency band is assigned to P = S(II) bond stretching vibration frequency. The origin of the higher frequency band P=S(I) is uncertain, but whatever its origin, its diagnostic value is beyond doubt. In the nitro-saligenin cyclic alkoxy/phenoxy compounds, Das et al[7] have observed the two bands in the region : 630 - 775 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 effected 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 shows any systematic shifts which reflects 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 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[3].

The P-O-C (alkyl) group is characterized by a strong absorption band whose frequency lies between 1010 - 1040 cm^{-1} while the band due to P-O-C (aryl) group is found in the region : 1235 - 1260 cm^{-1}; this band is always accompanied by a second absorption band which has been attributed to either the sym. str. of the P-O-C (aromatic) system or to a seperate P-O str. which is not so coupled. Thomas[3] strongly favours the latter explanation which is supported by the persistence of this band in both P-O-P and P-O-H 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' for the substituent groups, p.34, ref. 3) of the substituents. This band lies between 870 - 910 cm^{-1} for all compounds; the frequency range quoted is that of the strongest band in this region.

2.7.3 DISCUSSION ON M/S SPECTRA

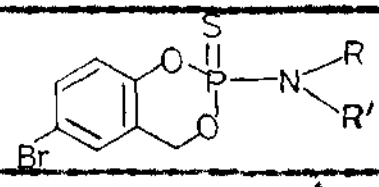
The mass spectra of these 6-bromo saligenin cyclic phosphoramidothionates have been analysed according to Cooks and Gerrard[8], Jörg et al[9], Damico et al[10,11], Gillis and Occolowitz[12], Djerassi, Budzikiewicz and Williams[13], and Shannon[14].

All compounds show parent molecular ion peaks(M^+) and $(M + 2)^+$ ion peaks. For the 6-bromo saligenin cyclic phosphoramidothionates, the $(M + 2)^+$ ion peaks are almost equal in intensity to the parent molecular ion peaks (M^+) because of the presence of a molecular ion containing the ^{81}Br isotope. Again all the bromine containing fragments show [fragment+2]$^+$ion peaks and fragment ion peaks of equal intensity. The result prove the presence of one bromine atom in the 6-bromo saligenin cyclic phosphoramidothionates.

In all cases fragmentation by loss of •SH radical from the molecular ion is important. The base peaks of the different phosphoramidothionates are tabulated in the following table.

Table 2.2

Base peaks of the 6-bromo saligenin cyclic phosphoramidothionates



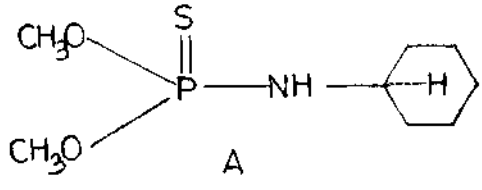
Alkylamido group	Code No. of the compound	Base peak (m/z)	Ion
Diisobutylamido	BF-6	348	$C_{12}H^+_{26}NO_2PSBr$
Dipentylamido	BF-8	43	$C_3H^+_7$

Contd...

Table 2.2(contd.)

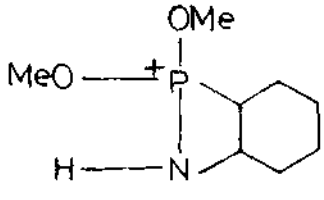
Alkylamido group	Code No. of the compound	Base peak (m/z)	Ion
Dibutylamido	BR-10	358	$(M - SH)^+$
2,6-Dimethylmorpholino	BR-12	362	$C_{12}H^+_{14}NO_3PSBr$
2-Ethylpiperidino	BR-14	346	$C_{12}H^+_{14}NO_2PSBr$
4-Benzylpiperidino	BR-15	91	$C_7H^+_7$
Hexamethyleneimido	BR-17	328	$(M-SH)^+$

Cooks and Gerrard[8] reported that compounds of the type (A) formed the base peak by loss



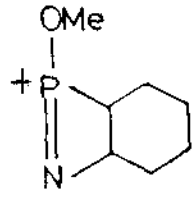
A

of •Sn radical directly from molecular ion. By deuteration of the methyl group and the amino group it was shown that the hydrogen of the Sn is abstracted from the cyclohexyl ring and not from the N-H entity. There was no preliminary hydrogen shift to sulphur. They postulated the structure (B) for the product ion.



B

By specific loss of the amino hydrogen, (B) further lost CH_3CH giving an ion for which they postulated structure (C).



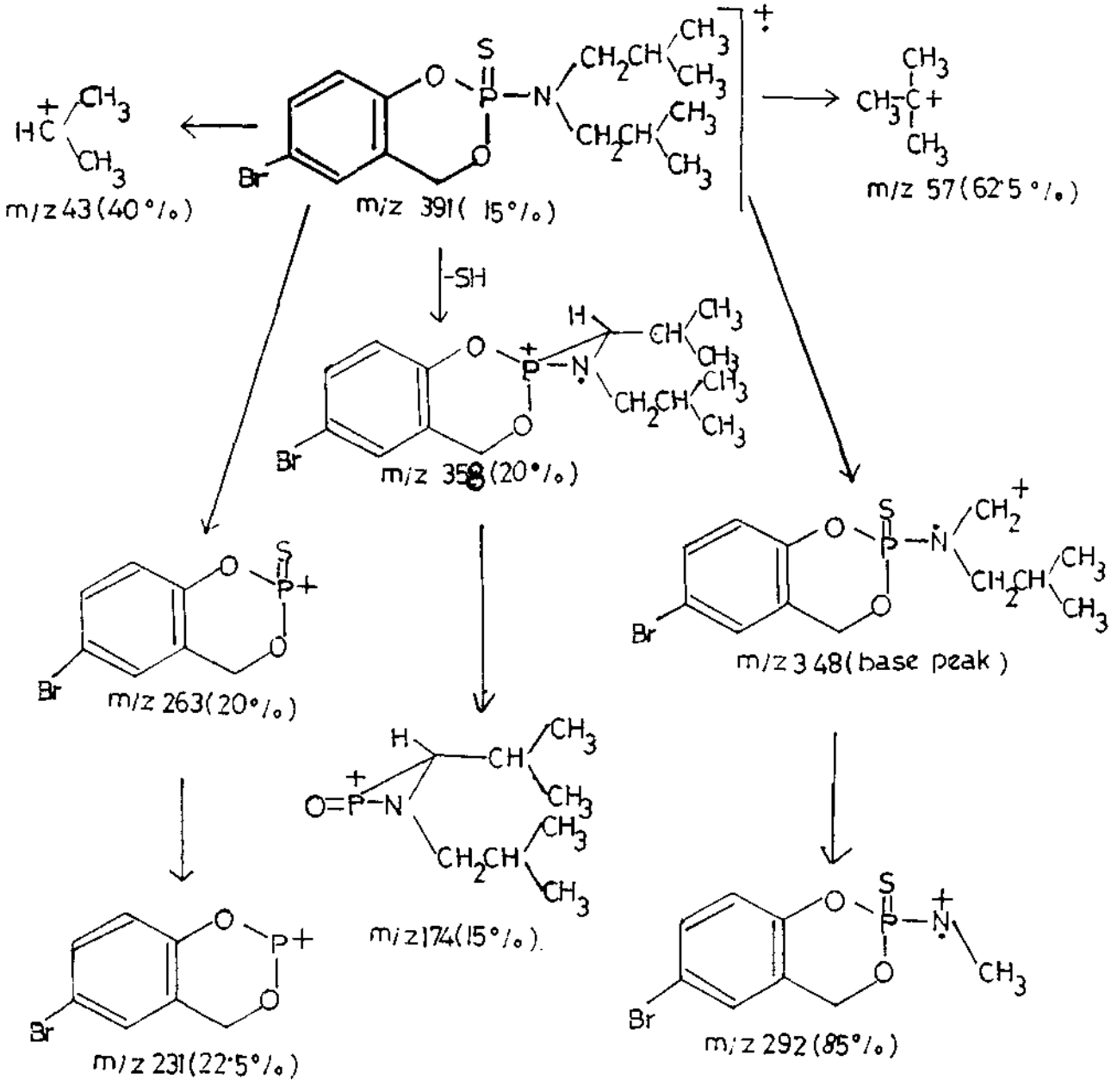
C

These two structures (Band C) are supported by analogous fragmentations of the related compounds.

Following Cooks and Gerrard[8], we postulate the mass fragmentation process for the different bromo saligenin cyclic phosphoramidothionates.

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, scheme 1). 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 19) is formed by the loss of C_7H_9BrO 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 37, %RI 62.5) is formed by the elimination of $C_{11}H_{14}NO_2PSBr$ 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.

Dipentylamido compound (BR-8) shows m/Z 43 ion as the base peak by the elimination of $C_{14}H_{20}NO_2PSBr$ from the molecular



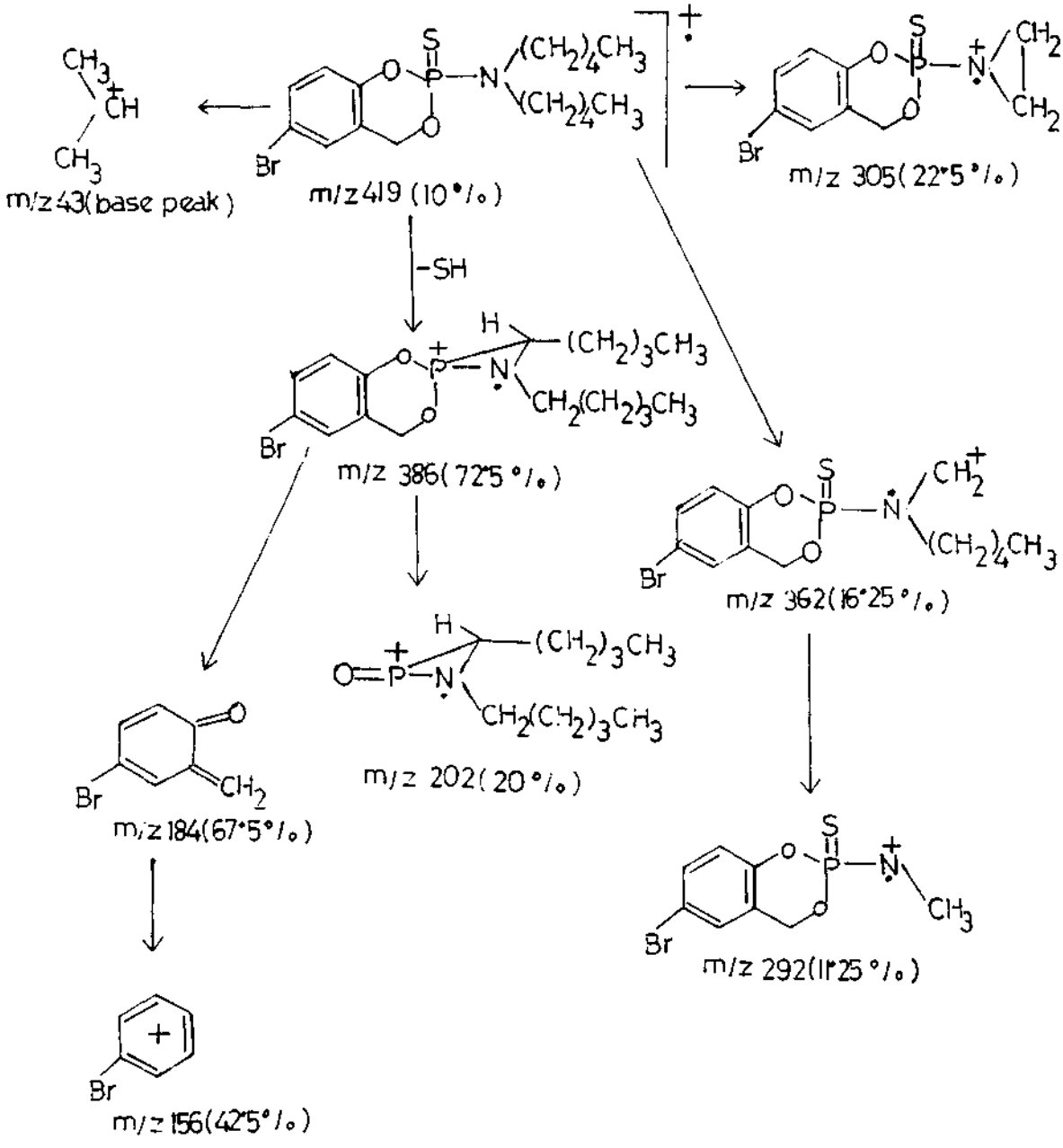
SCHEME I. PRINCIPAL MODES OF MASS FRAGMENTATION PROCESS OF BR-6

ion (m/Z 419, % RI 10, scheme II). The ion (m/Z 386, %RI 72.5) is formed by the direct loss of SH from the molecular ion.

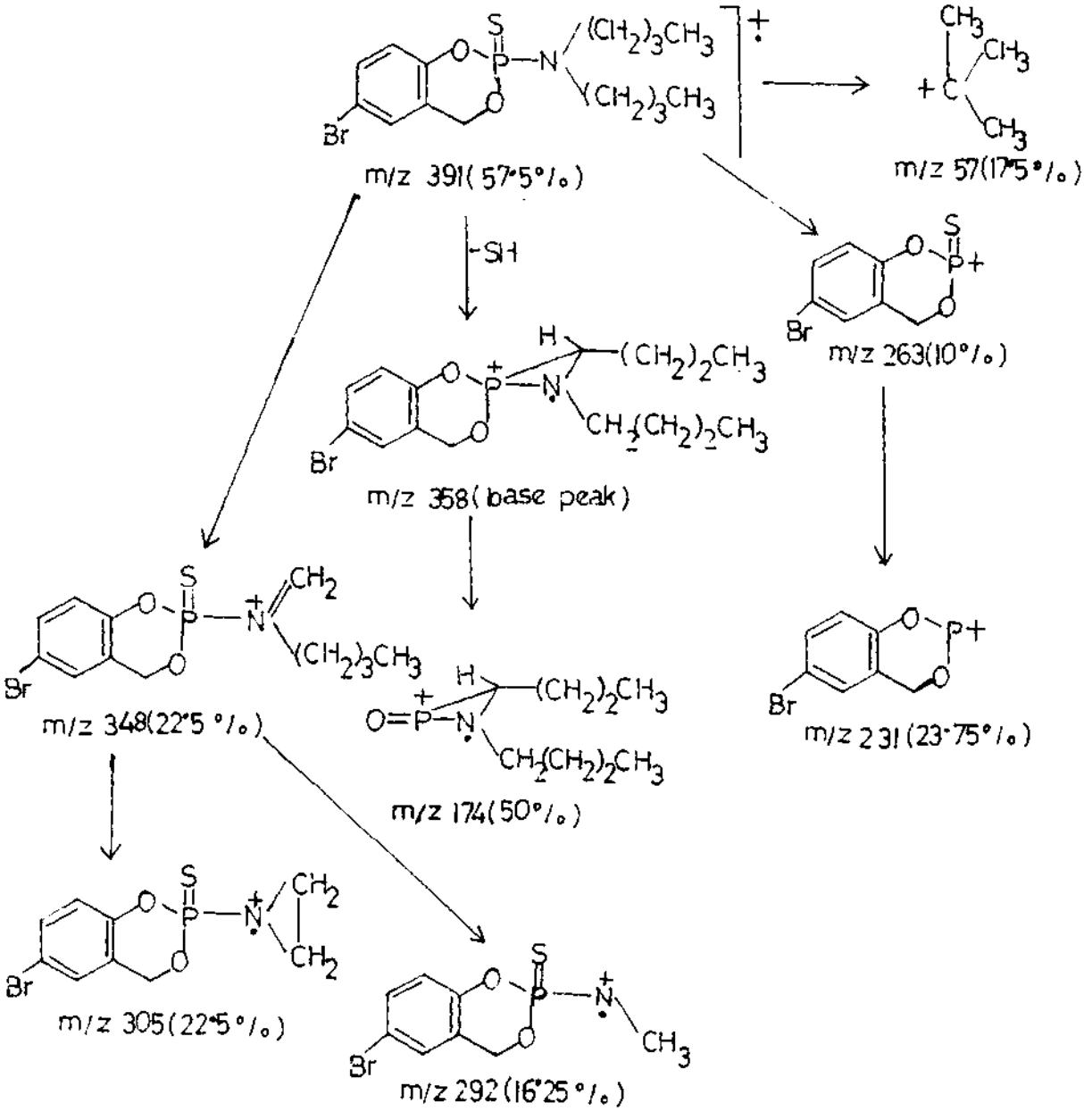
The ion (m/Z 202, %RI 20) is formed by the loss of C_7H_5BrO from the ion, m/Z 386. The ions (m/Z 305, %RI 22.5) and (m/Z 362, %RI 16.25) are formed from the molecular ion by the loss of C_8H_{18} and C_4H_9 respectively. The ion (m/Z 292, %RI 11.25) is formed by the elimination of C_9H_{10} from the ion m/Z 362. The ions (m/Z 184, %RI 67.5 and m/Z 156, %RI 42.5) have also been observed.

In the spectrum of dibutylamido compound (BR-10), the base peak is due to m/Z 358 ion, (scheme III) 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 30) is formed by the loss of C_7H_5BrO from the ion, m/Z 358. The ion (m/Z 37, %RI 17.5) is formed by the elimination of $C_{11}H_{14}NO_2PSBr$ from the molecular ion. Theions (m/Z 263, %RI 10 and m/Z 231, %RI 23.75) have also been observed.

In the case of 2,6-dimethylmorpholino compound (BR-12), the base peak is due to m/Z 362 ion (scheme IV), formed from the molecular ion (m/Z 377, %RI 22.61) by the elimination of CH_3. The ion (m/Z 344, %RI 11.9) is formed by the elimination of SH



SCHEME II. PRINCIPAL MODES OF MASS FRAGMENTATION PROCESS OF BR-8



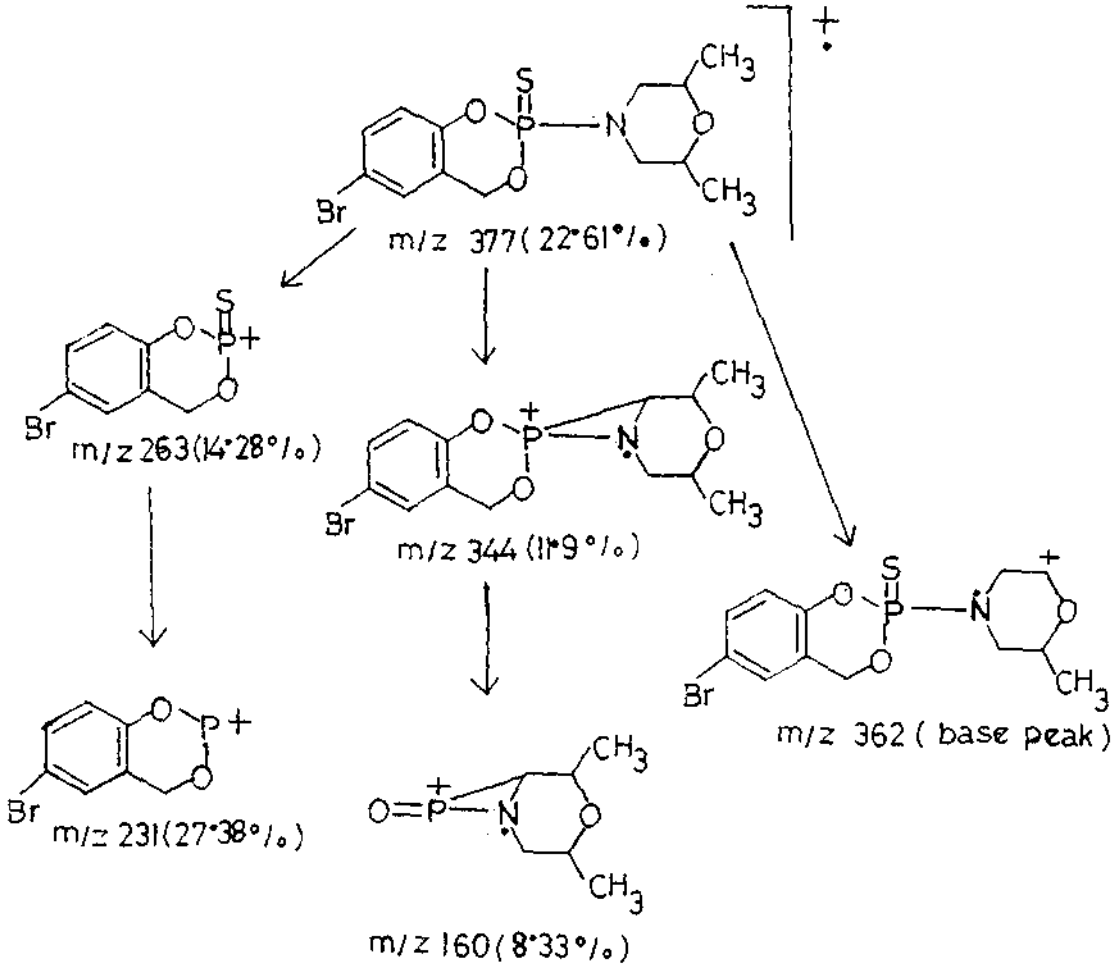
SCHEME III. PRINCIPAL MODES OF MASS FRAGMENTATION PROCESS OF BR-10

from the molecular ion. The ion (m/Z 160, %RI 8.33) is formed by the elimination of C_7H_3BrO from the ion, m/Z 344. The ion (m/Z 263, %RI 14.28)is formed by the loss of $C_6H_{12}NO$ from the molecular ion. The ion (m/Z 231, %RI 27.38) is formed from the ion, m/Z 263, by the elimination of 'S' radical.

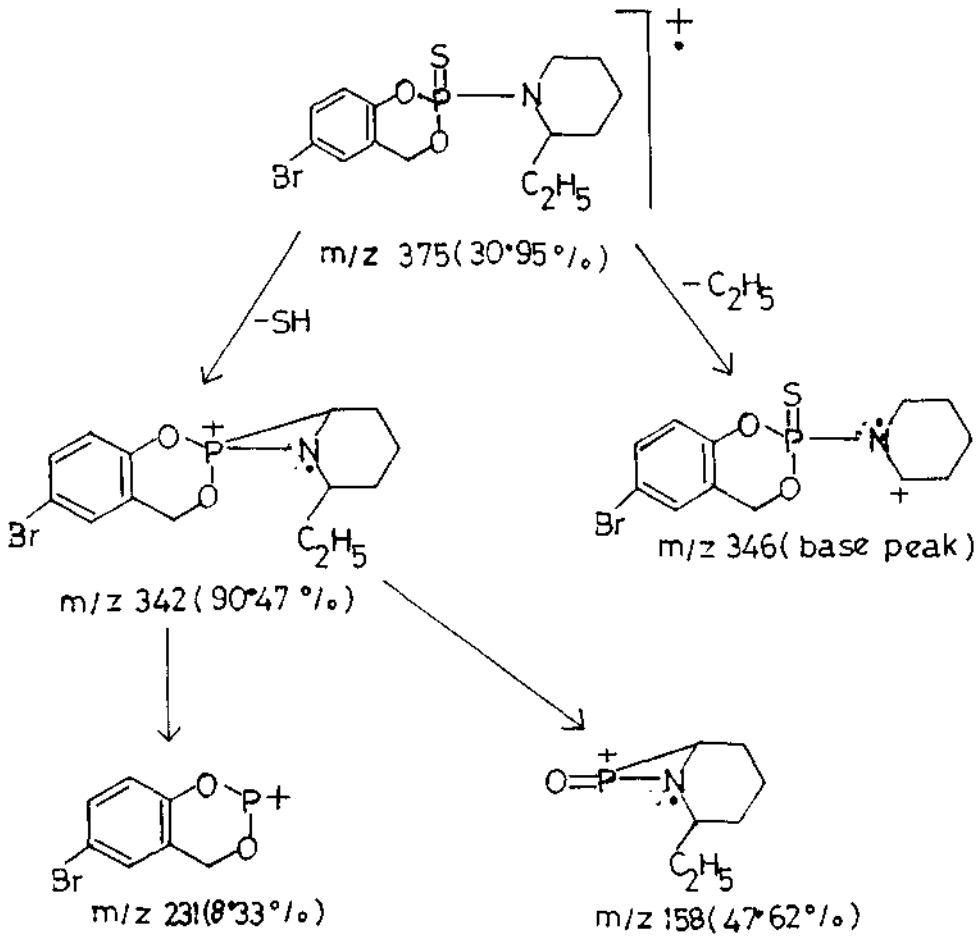
In the spectrum of 2-ethylpiperidino compound (BR-14), the base peak is due to m/Z 346 ion (scheme V), and is formed by the elimination of C_2H_5 from the molecular ion, m/Z 375. The ion (m/Z 342, %RI 90.47) is formed by the loss of SH from the molecular ion. The ion (m/Z 138, %RI 47.42) is formed by the elimination of C_7H_3BrO from the ion, m/Z 342. The ion (m/Z 231, %RI 8.33) is formed by the elimination of $C_7H_{13}N$ from the ion, m/Z 342.

In the spectrum of 4-benzylpiperidino compound (BR-15), the base peak is due to m/Z 91 ion (scheme VI) formed by elimination of C_5H_9N from the ion (m/Z 174, %RI 16.66) which is formed from the molecular ion (m/Z 437, %RI11.31) by the loss of $C_7H_3O_2HSBr$. The ion (m/Z 404, %RI 80.95) is formed by the elimination of SH from the molecular ion. The ion (m/Z 220, %RI 21.42) is formed by the elimination of C_7H_3BrO from the ion, m/Z 404. The ion (m/Z 231, %RI 9.52) has also been observed.

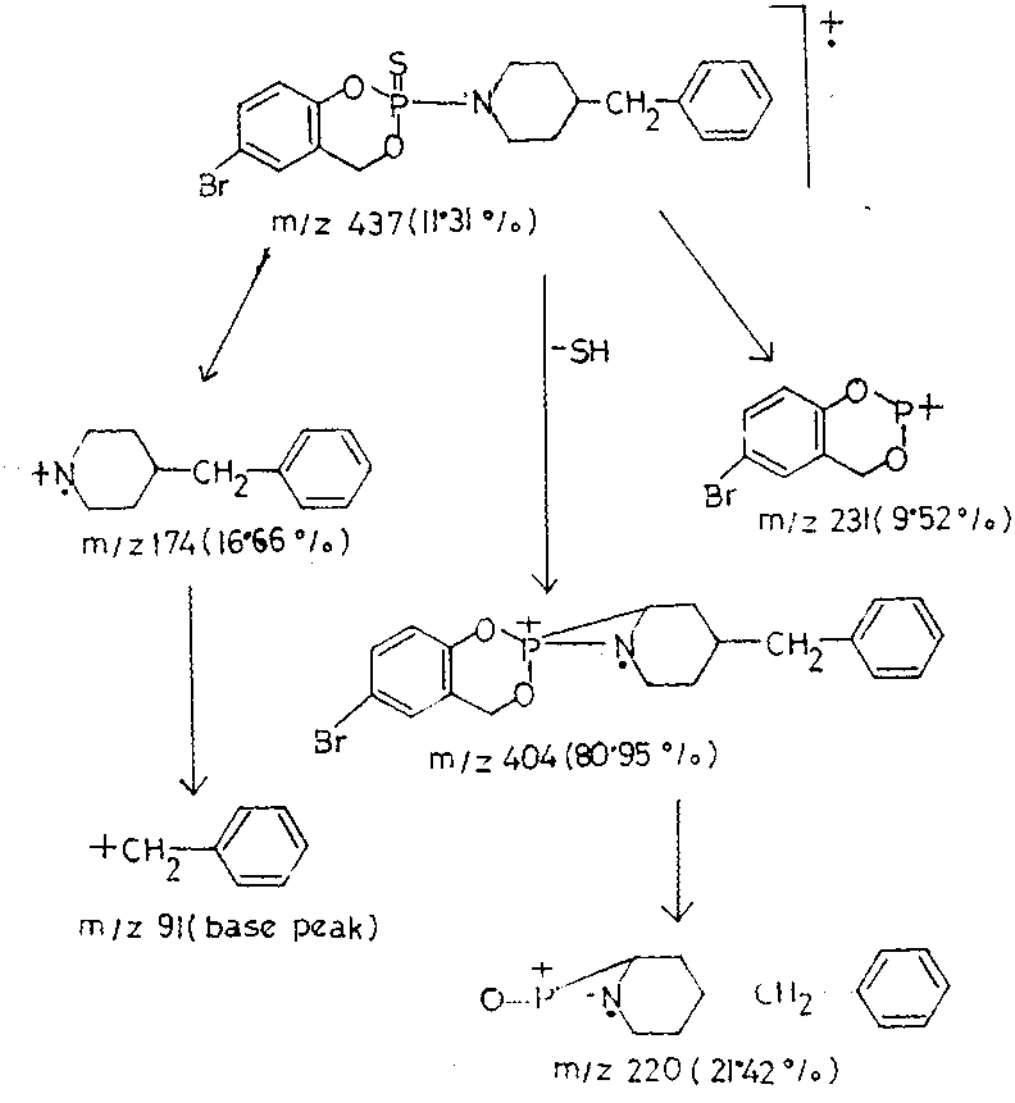
In the spectrum of hexamethylenimido compound (BR-17) the base peak is due to m/Z 328 ion (scheme VII) formed by the direct elimination of SH from the molecular ion (m/Z 361, %RI54.76). The ion (m/Z 144, %RI 57.14) is formed by the elimination of C_7H_3BrO from the ion, m/Z 328. The ion (m/Z 98, %RI 16.66) is formed by



SCHEME IV. PRINCIPAL MODES OF MASS FRAGMENTATION PROCESS OF BR-12

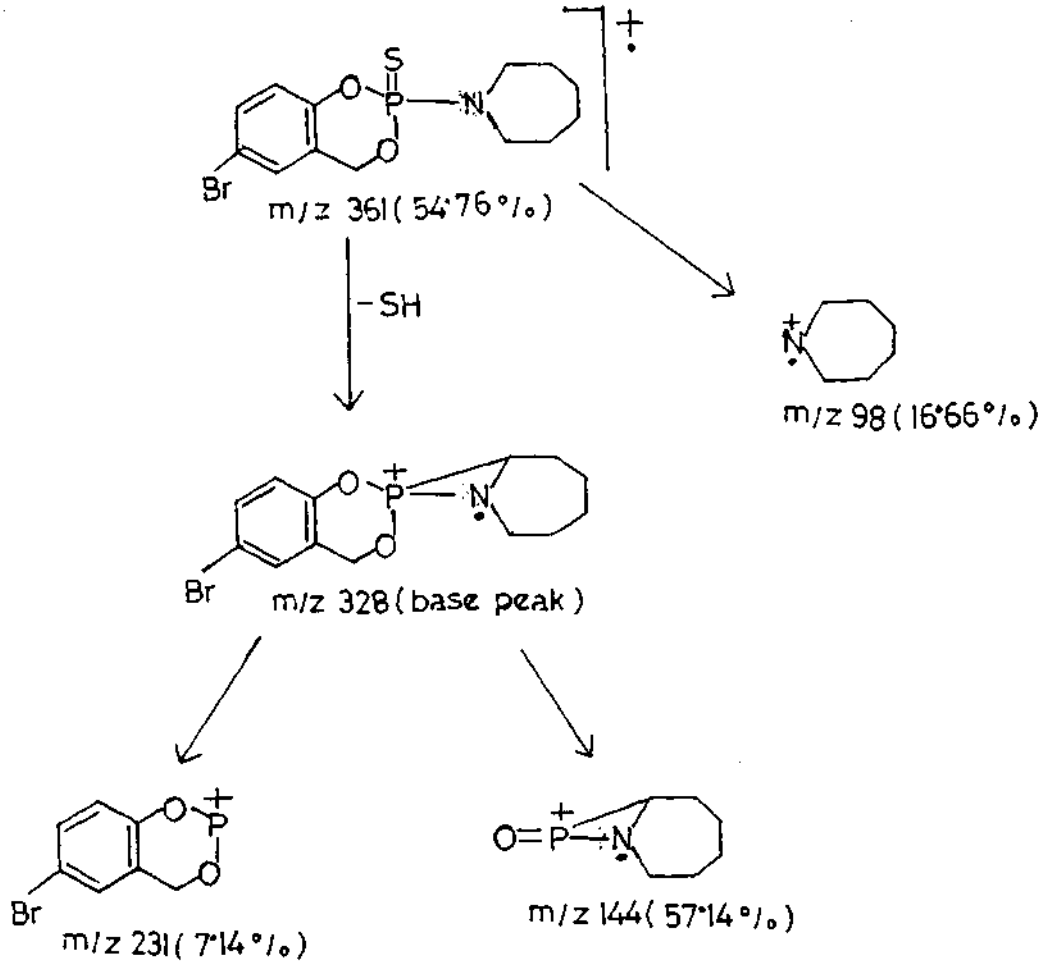


SCHEME V. PRINCIPAL MODES OF MASS FRAGMENTATION PROCESS OF BR-14



SCHEME VI. PRINCIPAL MODES OF MASS FRAGMENTATION PROCESS OF BR-15

the loss of $C_7H_{13}O_2$ from the molecular ion. The ion (m/Z 231, fig 7.14) is formed by the elimination of $C_6H_{11}N$ from the ion, m/Z 32[illegible].



SCHEME VII. PRINCIPAL MODES OF MASS FRAGMENTATION PROCESS OF BR-17

2.74 DISCUSSION ON NMR SPECTRA

For the proton noise decoupled ^{13}C spectra of BR-6, BR-12, BR-14 and BR-17 at 100 MHz the saturated carbons 2 and 3 bonds from the phosphorus show splitting due to $^{13}C - ^{31}P$ spin coupling. The chemical shift values for the CH_2 carbon (C_4) in the dioxaphosphorin ring of BR-6, BR-12, BR-14 and BR-17 are almost the same. ^{13}C chemical shifts are often quite sensitive to conformation, it supports the similarity of the structure.

Das[15] reported that in compound BD-15 (2-pyrolidino-6-nitro-4H-1,3,2-benzodioxaphosphorin 2-sulphide), the plot expansion of aliphatic region of ^{13}C spectrum (Fig.2.39) reveals the doubling of lines due to the spin of the ^{31}P nucleus. Fig.40 is a plot expansion of aromatic region of the ^{13}C spectrum of BD-15. Four of the ring carbons show measurable coupling to the ^{31}P nucleus.

These couplings are :

Carbon	Coupling constants (Hz)
C_8	8.19
C_{10}	12.38
C_9	7.30
C_7	0.93

For the aromatic region of ^{13}C spectrum of BR-6, BR-12, BR-14 and BR-17 the chemical shift values and coupling constants are in close agreement with that of BD-15.

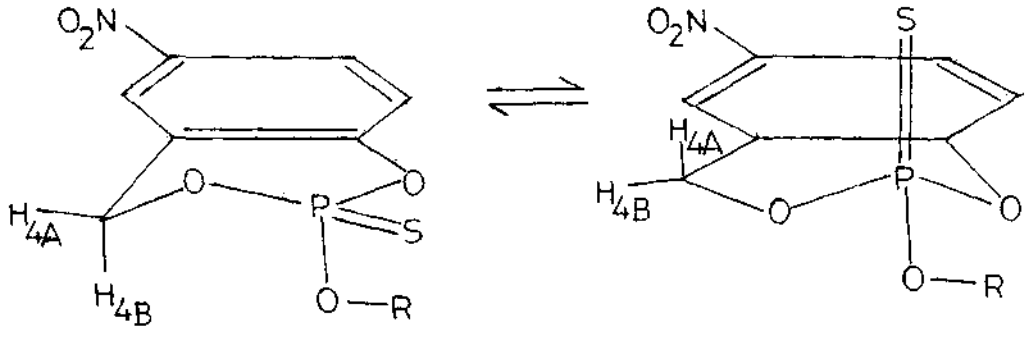
The ^1H NMR spectra for all the compounds have signals at $\delta = 4.63 - 6.74$ ppm for the $-CH_2-$ group protons in the dioxaphosphorin ring and for all the compounds the signal is a eight line multiplet.

The ^1H NMR spectra of several 2-alkoxy/phenoxy/alkylamido-6-nitro-4H-1,3,2-benzodioxaphosphorin 2-sulphides have been reported by Das[15].

The ^1H NMR signal for the $-CH_2-$group protons in dioxaphosphorin ring in BD-10 (2-cyclohexylamido-6-nitro-4H-1,3,2-benzodioxaphosphorin 2-sulphide) is a eight line multiplet at $\delta = 5.4$ ppm (Fig.40). The Fig.41 and Fig.42 are the ^1H NMR spectra of BD-8 (2-ethoxy-6-nitro-4H-1,3,2-benzodioxaphosphorin 2-sulphide) and BD-9 (2-phenoxy-6-nitro-4H-1,3,2-benzodioxaphosphorin 2-sulphide) respectively. The signal at $\delta = 5.4$ ppm in BD-8, in BD-9 and in BD-10 are different from each other. If we study the ^1H NMR spectra of BD-8, BD-9 and BD-10 (Fig.2.41,2.42 and2.40) in the given order, it is fairly evident that the chemical shift difference of the protons H_{4A} and H_{4B} is increasing in going from BD-8 to BD-10. Also, the 2-substituent is at the same time increasing in bulk, and probably spending more time in the conformation with the least steric interactions. In BD-10 this may be mainly in one conformation; while in BD-8 it appears that both the chemical shift difference and the difference between $^3J_{P-O-C-H_{4A}}$ and $^3J_{P-O-C-H_{4B}}$ are quite small. This suggests that the molecule exists as an average of the two conformations with rapid interconversion

between them. In case of BD-9 it seems that an intermediate situation prevails.

In either of the conformation I and II one of the couplings, $^3J_{P-O-C-H_{4A}}$ or $^3J_{P-O-C-H_{4B}}$ will be large and other one will be



I II

small, since the $P-O-C-H_4$ dihedral angle for one of them is close to 180° and for the other it is close to 60°. The plot expansion of the 5.0 - 5.8 ppm region for BD-10 (Fig.44) shows couplings of 3.6, 1.0, 6.3, 14.7 and 25.6 Hz. Since the 14.7 Hz coupling is certainly the geminal $^2J_{H_A - H_B}$ coupling constant, this then leaves 6.3 and 25.6 Hz as the two $^3J_{P-O-C-H}$ coupling constants. Consequently we can conclude that BD-10 is probably mainly in one conformation. On the other hand, the pattern found in BD-8 can only be explained by nearly equal (but not exactly) coupling constants and a small chemical shift difference in H_A and H_B, which requires rapid interconversion to give an average of the coupling constants and chemical shifts. BD-9 seems to be an intermediate case.

In BD-10, the small couplings seen in Fig.44 arise from coupling to the aromatic ring protons H_5, H_7 and H_8. The conforma-

tion II seems less hindered for bulky substituent groups such as those in BD-9 and especially in BD-10. Therefore, let us assume that in BD-10 conformation II predominates. In that case the dihedral angle of 180° belongs to H_B, and we can identify it as the up field group of lines showing equal coupling of 0.6 Hz to the three aromatic protons, H_B, H_E and H_D. Proton H_A is more strongly coupled to H_D and H_F with J = 1.0Hz, but not to H_B. This can be confirmed by spin decoupling experiments shown in Fig.45 and Fig.46. In Fig.45 aromatic protons H_D and H_F were irradiated at 6.1 ppm and the triplet structure of H_A disappeared; the quartet structure of H_B also collapsed due to irradiation of H_D and H_F, which removed the effects of their spins, but the residual line width masks the remaining small coupling to H_D. However in Fig.46, in which only H_D was irradiated, we can see that the quartets have become triplets as expected.

The plot expansion of the aromatic region (Fig.47 for BD-10) shows the basic ABX pattern with additional long range couplings to H_D and H_F. The interpretation of this spectral region is facilitated by examining Fig.48 in which the $-CH_2-$ group ms in the dioxaphosphorin ring was decoupled. In Fig.48, H_F appears as an eight line pattern. In Fig.47, each line of this pattern is split by the protons H_{4A} and H_{4B} into triplets with 0.8 spacings. This is the average of the actual couplings ($J_{H_F - H_{4A}}$ = 1.0 Hz; $J_{H_F - H_{4B}}$ = 0.6 Hz), due to the fact that H_A and H_B are strongly coupled ($^2J_{H_A - H_B}$ = 14.7Hz) and separated by a small chemical shift.

Our compound behaves like BO-10. The ^{1}H NMR spectra of the phosphoramidothionates and ^{13}C spectra of some of these compounds seem to be in general accord with the structure II although they do not preclude another conformation (i.e., its inverted form, where oxygen and phosphorus lie below the plane of the aromatic ring). Further studies including X-ray crystal structure determination are in progress. However, it may be pointed out that the structure II appears to explain, to a reasonable extent, the reversal of the expected proton chemical shift order for the quasi-axial and quasi-equatorial protons, due to the position of the magnetically anisotropic P=S bond related to the $-CH_2-$group in the dioxaphosphorin ring.

CHAPTER 3

BIOLOGICAL ACTIVITIES AND CHEMICAL INTERCONVERSIONS OF SOME 2-ALKYLAMINO-4-OXO-SALICENIN CYCLIC HYDROPHOSPHATES

Chapter - 3

BIOLOGICAL ACTIVITIES AND CHEMICAL HYDROLYSIS OF SOME 2-ALKYLAMIDO-6-BROMO SALIGENIN CYCLIC PHOSPHORAMIDOTHIONATES

MATERIALS AND METHODS :

3.1. ORAL INSECTICIDAL ACTIVITY ON COCKROACHES

Insecticidal tests were performed on the American cockroach, *Periplaneta americana* (Linn) according to Busvine[16] with minor modifications. Adults of *P. americana* weighing about 1 g to 1.2 g were collected in the month of June - July, 1985 from a particular location of the North Bengal University campus, where they were never exposed to any organophosphorus insecticides. For preliminary experiment, ten cockroaches in each pot were exposed to different dosages of the compounds in dry sugar bait and after 48 h the mortality was determined, and the approximate minimum concentration required for 100 per cent mortality (LC_{100}, $\mu g/g$ basis) was found out.

To determine more precise LC_{100} (the minimum concentration required for 100 per cent mortality) value of each compound, one cockroach of known weight in each pot was exposed to known quantity of the phosphoramidothionates progressively increasing its concentration by 2 $\mu g/g$; for salithion the concentration was increased

by 1 μg/g. Each experiment was triplicated and the average LC_{100} value was found out by using the simple aritmetical procedure[37]. Before conducting the experiments on them, the cockroaches were kept starved for 24 h. However, the varying susceptibility of male and female cockroaches to different compounds were ignored during the experiment.

3.2. ACUTE ORAL TOXICITY OB RATS

Oral toxicity testing was conducted on 6-12 months old male white albino rats, weighing 140-200 g, each housed in separate compartments of a cage. All animals had free access to food and water. Different doseges of a compound were mixed with boiled fish and given to the animals at their habitual[38] feeding time. The mortality within 48 h were recorded along with the toxic symptoms. Acute oral toxic dosage was found out by varying the amount of compound proportionately. The negligible amount of compound wasted by the animal during dieting was roughly accounted for in determining the dosage.

3.3. ANTICHOLINESTERASE ACTIVITY

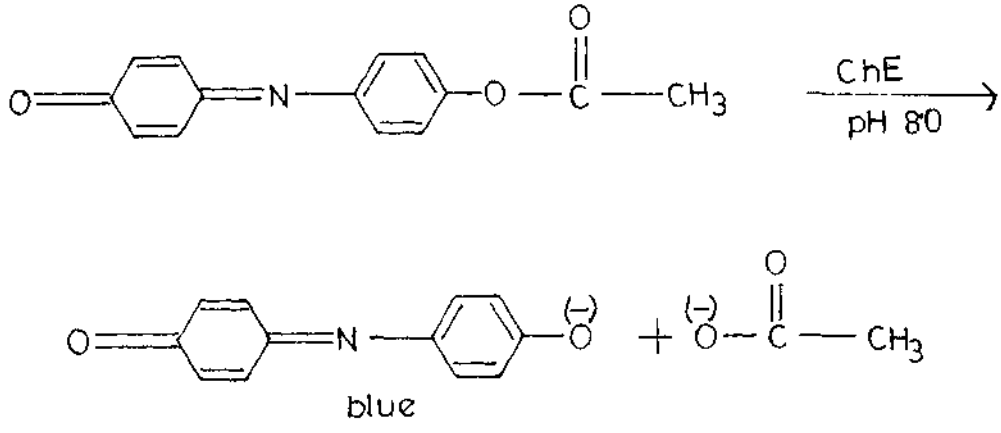
The organophosphorus compounds have a common pharmacological property which is the ability to inhibit the activity of a group of enzymes, especially acetylcholinesterase (AChE), involved in the hydrolysis of esters of choline. Since these enzymes present widely in insects and mammals, many of the organophosphorus com-

pounds, used as insecticides also exhibit high mammalian toxicity.

There are four major methods for assaying cholinesterase activity and effect of inhibitors. The methods are potentiometric, titrimetric, manometric and colorimetric.

3.3.1 Anticholinesterase Activity in Blowfly-head homogenate

The acetyl cholinesterase inhibition of blowfly-head brie (BFAChE) has been measured by colorimetric method of Kramer and Gunson[19], using indophenyl acetate as an internal substrate-indicator in 0.05 M phosphate buffer of pH 8.0. The enzymatic reaction for indophenyl acetate is as follows :



(λ_{max} 625 nm.)

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

Reagents

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

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

(iii) Glycerol solution :

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

(iv) Saline solution :

9 g of NaCl was dissolved in 1 litre distilled water.

(v) Salt solution :

2.03 g of manganous chloride and 2.15 g of NaCl were dissolved in 250 ml of water.

(vi) Preparation of working solution of acetyl cholinesterase from blow fly-heads

About 500 blowfly (Chrysomyia megacephala) closed in a glass vessel were stored in a deep freeze for 1-2 h. They were then

transferred in a container, decapitated with a shaving blade and forceps. 250 heads were combined with 2 ml of salt solution and 2 g of washed sand in a prechilled size no. 1 mortar. The heads were slowly groud, then transferred to 50 ml plastic centrifuge tube with one 3 ml aliquots of cold saline solution and two 5 ml aliquots of buffer solution. The head fragments were removed by centrifugation for 10 minutes at 10,000 r.p.m. in super speed centrifuge at 4°C. The supernatant liquid was ~~ex~~ decanted into a graduated cylinder and the fragmented heads were mixed with 10 ml buffer solution and centrifuged again at 10,000 r.p.m. This extraction procedure was repeated twice. The supernatant solutions were combined and the volume was adjusted to 200 ml with buffer solution so that each ml was equivalent to 1 fly head. This solution was stocked frozen in deep freeze. One ml of this solution was diluted to 5 ml with buffer solution so that each 5 ml of the diluted solution contained single fly-head and used for each set of the experiment.

<u>Methods</u> :

A series of 15 pyrex beakers (numbered 1,2,3, ——— etc.) containing different amounts of inhibiters (different phosphoramidothionates) in acetone along with one marked 'control' without inhibiters were arranged. 0.5 ml of glycerol solution was poured in each of the beaker including the 'control'. The acetone in the beaker was removed by blowing cold air.

To the 'control' 5 ml of working enzyme buffer solution was added and simultaneously the stop watch was started. At the interval of exactly 2 minutes, 5 ml of the enzyme-buffer solution was added to each of the remaining beaker. After exactly 30 minutes, 0.15 ml of the indophenylacetate solution (3.3×10^{-3}M) was added to the beaker marked 'control' and subsequently to each beaker of the series at the interval of 2 minutes and then kept to be incubated at 30°C. After incubation for exactly 30 minutes the absorbances of 'control' and remaining solutions were successively noted in the spectrophotometer at 625 nm with reference to enzyme-buffer (reagent blank) solution.

<u>Calculation</u> :

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

3.3.2 <u>Antiacetylcholinesterase activity in Goat whole blood</u>

The method employed to determine the inhibition of the activity of acetyl cholinesterase in goat whole blood by organophosphorus compounds was determined by colorimetric method of Kramer and Gmason[29], using indophenyl acetate as an internal substrate indicator in 0.05 M phosphate buffer of pH 8.0. The reaction mixture contained 5 ml of enzyme buffer solution (4.8 ml phosphate buffer solution along with 0.2 ml goat whole blood) and 0.15 ml indophenyl acetate (total volume = 5.15 ml, concentration of indophenyl acetate in the reaction mixture $\sim 10^{-6}$M). The readings of control and 'sample' were taken at 625 nm, after exactly

30 minutes incubation.

Reagents :

(i) Goat whole blood

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

The other reagents e.g. (ii) indophenyl acetate (iii) phosphate buffer solution (iv) glycerol solution were prepared just as same as mentioned in 3.3.1.

Method

The anticholinesterase activity in goat whole blood plasma was determined by the same method as described in 3.3.1. The only exception was that the contents of the beaker was filtered through a 4.25 cm Whatman no. 1 filter paper. The absorbances were noted in the spectrophotometer at 625 nm with reference to the filtered enzyme-buffer solution and % inhibition was calculated in the same way as described in 3.3.1.

3.4 PHYTOTOXICITY TEST

Phytotoxicity testing was conducted according to Eto et al[20]. Acetone solution of the compounds mixed with fixed amount

of water containing 0.2% Triton-X was prepared. 5 ml of this aqueous suspension containing 200, 100 and 50 ppm of the compounds was poured into a petridish bottom covered with absorbent cotton. Ten seeds of rice (pusa 2-21 variety supplied by Rice Research Station, Chinsurah, West Bengal) were placed on the cotton and kept at room temperature (25 - 27°C) for five days. Occasionally 2-4 ml water was added in each petridish so that the seeds remained in moist condition. Each test was triplicated. Number of germination was counted after 5 days.

3.5 CHEMICAL HYDROLYSIS

The chemical hydrolysis studies were performed in 0.0095(M) NaOH solution in 50 per cent ethanol of pH 11.85 at 30°C. 6-bromo-saligenin cyclic phosphoramidothionates on hydrolysis produced 5-bromo saligenin. The rates of hydrolysis were monitored by following the rate of formation of 5-bromo saligenin spectrophotometrically in a Shimadzu UV-240 instrument at the wave lengths λ = 294, 298 and 300 nm. These wave lengths were chosen as the difference in optical densities of 5-bromo saligenin and any of the phosphoramidothionates at the same concentration was maximum at this region.

Determination of the hydrolysis constants of 6-bromo saligenin cyclic phosphoramidothionates involve the following stages :

(i) Determination of the molar extinction coefficients (ϵ_2) of 5-bromo-saligenin in the alkali solution (0.0095(M) NaOH in 50%

ethanol of pH 11.85):

Solution of 5-bromo-saligenin of known concentration in the alkali solution was made and the optical densities of the solution at $\lambda = 294$, 298 and 300 nm were measured; from the values of the optical densities and concentration the molar extinction co-efficients (ϵ_2) of 5-bromo saligenin at $\lambda = 294$, 298 and 300 nm were determined.

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

As the rate of hydrolysis of the 6-bromo-saligenin cyclic phosphoramidothionates were extremely slow in the alkali solution (pH 11.85), the optical densities (at $\lambda = 294$, 298 and 300 nm) of the alkali solution of any of the phosphoramidothionates was measured as soon as the alkali solution was added; the optical densities were assumed to be due to the phosphoramidothionates only. From these values of optical densities $(O.D)_1$ and the concentration of the phosphoramidothionates, the molar extinction coefficients of the phosphoramidothionates (ϵ_1) were determined.

(iii) Determination of the amount of the phosphoramidothionates (C_t) hydrolysed after a certain interval :

After 240 m of the addition of the alkali solution the optical densities $(O.D)_t$ of the mixture of unhydrolysed phosphora-

midothionates and the hydrolyzed products were measured at λ= 294, 298 and 300 nm.

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

$$C_t = \frac{(O.D)_2 - (O.D)_1}{\varepsilon_2 - \varepsilon_1}$$

(iv) Determination of the hydrolysis rate constants (K_{hyd}) of the 6-bromo saligenin cyclic phosphoramidothionates :

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

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

where, C_o = initial concentration of the 6-bromo saligenin cyclic phosphoramidothionates.

C_t = concentration of the compound hydrolyzed after time t.

3.6 FUNGICIDAL ACTIVITY

(a) Test organism :

Helminthosporium oryzae Breda de Haan; causal organism of brown leaf spot disease of rice.

(b) Culture medium :

Solid medium : Malt extract agar. 20 g malt (Difco) extract was boiled in water till dissolved. 20 g agar agar (Kolbe Japan) was added and boiled until agar was well dissolved. 0.05 g of Chloramphenol was suspended in 10 ml of 95% alcohol and added to the medium as antibacterial agent.

The volume of the medium was then made upto 1 litre by adding water. pH of the medium was adjusted to 6.5 by adding NaOH solution. Medium was sterilised at 15 p.s.i for 20 minutes.

(c) Growth inhibition method :

Growth inhibition studies were made by using poisoned food technique[21]. Acetone solution of suitablequantity of the phosphoramidothionates in sterile water containing 0.01 per cent Triton - X was incorporated into melted malt agar so as to get the desired concentration of the compounds in the media. The test medium was poured into the sterile petridishes and after solidification the 7 mm, 8 days old culture disc was placed aseptically at the centre of the petridishes. Three replications on each test with appropriate control under same conditions were maintained. The petridishes were incubated at 30 ± 1°C in dark.

Linear growth of the fungal discs were measured after 24, 48, 72 and 96 h interval. Per cent inhibition over control was calculated following the equation given by Vincent[22].

3.7 QUANTITATIVE STRUCTURE ACTIVITY RELATIONSHIP

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

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

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

arising out of molecular manipulation. Information theory has been applied to the total molecular graph to define two topological indices, information contents (IC) and structural information content (SIC) - which have been found to be well correlated with the biological activities of drugs/pesticides[23].

The detailed mathematical theory of the partitioning of the vertices (atoms) of a molecular graph to determine its embedded geometrical complexity is given in the following pages along with an illustrative example of the partitioning process. The first order topological information content of different graphs can be computed according to the formulation of Sarkar et al[26]. If G is a molecular graph with vertex set X(G) and A_i (i = 1, 2, 3 ——— K) is a partition of X(G), then the probability scheme is given by

$$A_1, A_2 \text{ ------ } A_K$$

$$P_1, P_2 \text{ ------ } P_K$$

where,

$$P_i = \frac{n_i}{n}$$

and n_i and n are the cardinalities of A_i and X(G) respectively. The information content (IC)[26] considering the class partition of vertices with respect to first order neighbour is given by

Shannon's formula[27].

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

The logarithm is taken at a base 2 to measure the information in bits.

There are 44 vertices in the graph representing a molecule of the compound NH-6 (Fig. 3.1). Out of the 44 vertices, 23 represents hydrogen atoms, 1 represents nitrogen atom, 1 represents sulphur atom, 1 represents phosphorus atom, 1 represents bromine atom, 2 represents oxygen atoms and 15 represents carbon atoms.

Since the graph is connected, each vertex of this graph is incident with at least one vertex on it. Actually each vertex representing a hydrogen atom is incident with just one other vertex of the graph. We see from the graph that all the 23 vertices representing hydrogen atoms are such that all of them are incident with vertex representing carbon atom. So the co-ordinate of each hydrogen is : (1^4)

Now there is one vertex representing nitrogen atom. The valency of nitrogen is five. The vertex representing that of nitrogen is incident with that of two carbon atoms by two single

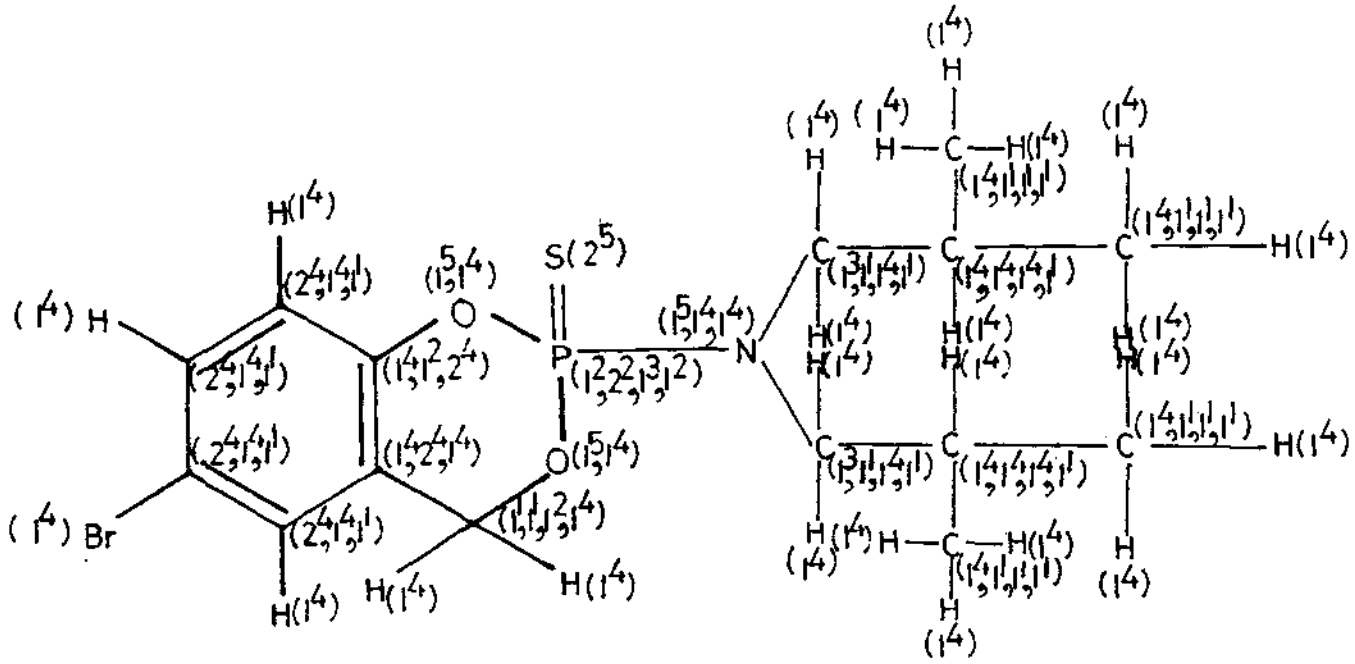


Fig. 3.1

bonds and with that of phosphorus by a single bond. The co-ordinate is given as :

$$(1^3, 1^4, 1^4)$$

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

The vertex representing that of phosphorus is incident with that of two oxygen atoms by two single bonds, a nitrogen by a single bond and to sulphur by a double bond. The co-ordinate is given as :

$$(1^2, 2^2, 1^3, 1^2)$$

The vertex representing that of bromine is incident with that of carbon atom by a single bond. So the co-ordinate is given as : (1^4)

There are two vertices representing that of oxygen. Two are similar, which are incident with the vertex representing carbon atom and posphorus atom. So the co-ordinate are given as :

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

The remaining 15 vertices representing carbon atoms have distinct co-ordinates which are :

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

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

Table 3.1

Partition class No. with co-ordinate	Number of atoms in the partitioned class	Probability P_i
$I(1^4)$	23	$23/44$
$II(1^3,1^4,1^4)$	1	$1/44$
$III(2^3)$	1	$1/44$
$IV(1^2,2^2,1^3,1^2)$	1	$1/44$
$V(1^4)$	1	$1/44$
$VI(1^3,1^4)$	2	$2/44$
$VII(2^4,1^4,1^1)$	4	$4/44$
$VIII(1^4,2^4,1^4)$	1	$1/44$
$IX(1^4,1^2,2^4)$	1	$1/44$
$X(1^1,1^1,1^2,1^4)$	1	$1/44$
$XI(1^2,1^1,1^4,1^1)$	2	$2/44$
$XII(1^4,1^4,1^4,1^1)$	2	$2/44$
$XIII(1^4,1^1,1^1,1^1)$	4	$4/44$

Therefore,

$I^1(C)$ = First order topological information content of H_2O

$$= -\left[1 \times \frac{23}{44} \log_2 \frac{23}{44} + 3 \times \frac{1}{44} \log_2 \frac{1}{44} + 2 \times \frac{4}{44} \log_2 \frac{4}{44} + \right.$$

$$7 \times \frac{1}{44} \log_2 \frac{1}{44}\Big]$$

$$= -\left[\frac{22}{44} \log_2 22 + \frac{8}{44} \log_2 2 + \frac{8}{44} \log_2 4 + \log_2 \frac{1}{44}\right]$$

$$= 2.5021 \text{ bits}$$

If the maximum of all possible information contents of Sh-6 corresponding to all possible modes partition of the set of vertices of the graph be represented by the symbol I(C)

$$I(C) = -\left[44 \times \frac{1}{44} \log_2 \frac{1}{44}\right]$$

$$= -\log_2 \frac{1}{44}$$

$$= 5.46 \text{ bits}$$

$$\therefore \text{ S.I.C.} = \frac{2.5021}{5.46} = 0.4583$$

$$\text{Rel.S.I.C.} = (1 - \text{S.I.C.})$$

$$= 0.5247$$

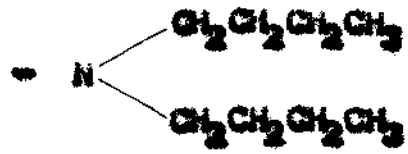
The S.I.C. of the other molecules have been calculated in the above method (given in Appendix-II).

(ii) <u>Determination of π and E</u>

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

An illustrative example for the estimation of (π) value is given in the following page. For the compound Sh-10 the substi-

tuent taken is dibutylamide

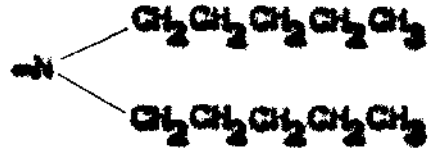


Thus, when the (π) values of $-CH_3$ = 0.89; $-CH_2$ = 0.46; chain length = -0.12 x (n - 1);

$$\pi = 2(-CH_3) + 6(-CH_2) - 0.12 \times 6$$
$$= 2(0.89) + 6(0.46) - 0.72$$
$$= 5.02$$

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

Taft's steric constant (E_s) are obtained from the Pomona Medicinal Chemistry Project Data Base[23]. An illustrative example for the estimation of (E_s) value is given below. For the compound SR-8, the substituent taken is dipentylamide group.



where the (E_s) values are

$-CH_2$ = -0.10

$-CH_3$ = -1.24

$$\therefore E_s = 2\left[4(-CH_2) + (-CH_3)\right]$$
$$= 2\left[4(-0.10) + (-1.24)\right]$$
$$= 2\left[(-0.40) + (-1.24)\right]$$
$$= 2(-1.64)$$
$$= -3.28$$

The (E_s) values for other molecules were calculated in the above method.

(iii) Determination of partition co-efficient

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

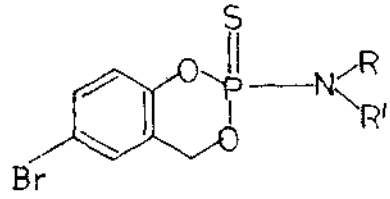
3.8. RESULTS AND DISCUSSION

3.8.1. INSECTICIDAL ACTIVITY

The oral insecticidal activity of the compounds against cockroaches, *P. americana* (Linn), are listed in table 3.2, and the results have been compared with sulfiphion, and 2-methoxy-6-

nitro-4H-1,3,2 - benzodioxaphosphorin 2-sulphide (BD-8).

Table 3.2



Alkylamide group	Code No.	Conc. showing 100% mortality (LC_{100}) µg/g
Diisobutylamide	BR-6	>100
Dipentylamide	BR-8	>100
Dihexylamide	BR-9	>100
Dibutylamide	BR-10	>100
2,6-Dimethyl-morpholine	BR-12	>100
2-Ethylpiperidine	BR-14	>100
4-Benzylpiperidine	BR-15	>100
Hexamethyleneimide	BR-17	>100
	BD-8	6
	Salithion	10

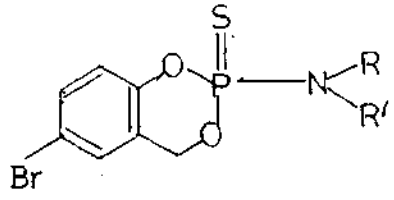
The data presented in the table 3.2 reveal that all phosphoramidothionates have less oral insecticidal activity than BD-8 and salithion; BD-8 has greater insecticidal activity than salithion;

6-bromo saligenin cyclic phosphoramidothionates are almost non-insecticidal.

3.8.2. ACUTE ORAL TOXICITY ON RATS

The acute oral toxicity data (LD_{50}) of the compounds on male rats are presented in table 3.3 and the results have been compared with salithion and 2-methoxy 6-nitro-4H- 1,3,2 - benzodioxaphosphorin 2-sulphide (SD-8).

Table 3.3



Alkylamide group	Code No.	LD_{50} (mg/kg) male rat.
Diisobutylamide	SR-6	>250
Dipentylamide	SR-8	>250
Dihexylamide	SR-9	>250
Dibutylamide	SR-10	>250
2,6-Dimethylmorpholine	SR-12	<250
2-Ethylpiperidine	SR-14	<250
4-Benzylpiperidine	SR-15	>250
Hexamethyleneimide	SR-17	<250
	SD-8	120 - 135
	Salithion	102

Data presented in the table 3.3 show that all compounds are less toxic than salithion. SR-12, SR-14 and SR-17 have greater toxicity to rats compared with other phosphoramidothionates. Before death the rats were found to suffer from acute respiratory trouble. In some cases, a fluid with blood-stain oozed out of nostrils and eyes of the rats.

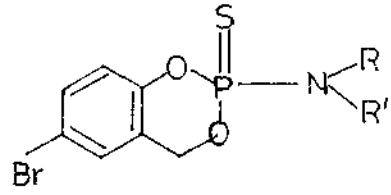
The LD_{50} values given here are obtained from only preliminary experiments; further works are required for the determination of the accurate LD_{50} values. However the relative toxicity of the compounds to male white albino rats can be judged from the values.

3.8.3. ANTICHOLINESTERASE ACTIVITY

The anticholinesterase activity data of 6-bromo saligenin cyclic phosphoramidothionates for blow-fly head homogenate (BFAChE) and goat whole blood (AChE) are listed in experiments 1 to 16 (pages 136 to 151); the molar I_{50} values of these compounds, calculated from the regression analysis are summarized in table 3.4.

Table 3.4

Anticholinesterase activity on blow-fly head homogenate (BFAChE) and goat whole blood (AChE)



Alkylamide group	Code No.	$I_{50}(M) \times 10^3$ (BFAChE) Blow-fly	$I_{50}(M) \times 10^3$ (AChE) Goat whole blood
Diisobutylamide	BR-4	9.84	28.93
Dipentylamide	BR-8	2.35	14.03
Dihexylamide	BR-9	2.00	1.15
Dibutylamide	BR-10	3.15	1.22
2,6-Dimethylmorpholine	BR-12	5.14	28.24
2-Ethylpiperidine	BR-14	1.97	0.70
4-Benzylpiperidine	BR-15	4.47	32.90
Hexamethyleneimide	BR-17	2.40	4.09

It has been observed that for the phosphoramidothionates (BR-4, BR-8, BR-12, BR-15 and BR-17), the BFAChE is more inhibited than

the AChE. On the other hand, for the phosphoramidothionates (BR-9, BR-10 and BR-14), the AChE is more inhibited than the BFAChE.

The $I_{50}(\mu M)$ value of the 6-bromo saligenin cyclic phosphoramidothionates for BFAChE increases in the following order :

BR-9 ≈ BR-14 < BR-8 ≈ BR-17 < BR-10 < BR-15 < BR-12 < BR-6 i.e., the dibenzylamido compound (BR-9) and 2-ethylpiperidinecompound (BR-14) are most active and diisobutylamido compound (BR-6)is least active.

The $I_{50}(\mu M)$ value of the 6-bromo saligenin cyclic phosphoramidothionates for AChE increases in the following order :

BR-14 < BR-9 ≈ BR-10 < BR-17 < BR-8 < BR-6 ≈ BR-12 < BR-15
i.e., the 2-ethylpiperidine compound (BR-14) is most active and 4-benzylpiperidine compound (BR-15) is the least active.

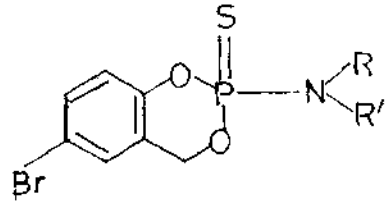
To sum up 2-(2-ethylpiperidino) 6-bromo-4H-1,3,2- benzodioxaphosphorin 2-sulphide (BR-14) has highest antiacetylcholinesterase activity for BFAChE and AChE.

3.8.4. PHYTOTOXIC PROPERTIES

The phytotoxicity data against rice seeds (pusa 2-21 variety) for the phosphoramidothionates are listed in table 3.5.

Table 3.5

The effects of phosphoramidothionates on germination of rice seed (pusa 2-21 variety)



Alkylamide group	Code No.	Percent germination of seeds at different conc.		
		200 ppm	100 ppm	50 ppm
Diisobutylamide	BR-4	90	100	100
Dipentylamide	BR-6	80	100	100
Diamylamide	BR-9	90	100	100
Dibutylamide	BR-10	80	100	100
2,6-Dimethyl-morpholine	BR-12	100	100	100
2-Ethylpiperidine	BR-14	90	100	100
4-Benzylpiperidine	BR-16	90	100	100
Hexamethyleneimide	BR-17	90	100	100

At 100 ppm and 50 ppm none of the compounds is phytotoxic to rice seed (pusa 2-21 variety); at 200 ppm the compounds except BR-12 show slight phytotoxicity. In case of the compounds BR-6,

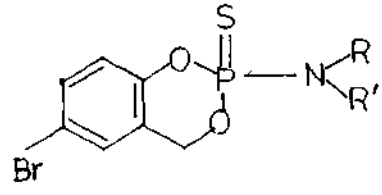
BR-9, BR-14, BR-15 and BR-17, at 200 ppm 90 per cent germination are observed and for the compounds BR-8, BR-10, at 200 ppm 80 per cent germination are observed.

3.8.5. CHEMICAL HYDROLYSIS

The chemical hydrolysis of 6-bromo saligenin cyclic phosphoramidothionates were carried out in 0.0095(M) NaOH solution in 50 per cent ethanol of pH 11.85 at 30°C at λ = 294, 298 and 300 nm. Three sets of experiments in each case have been performed. The hydrolysis data for each compound at λ=294, 298 and 300 nm and the value of K_{hyd} calculated thereof for a particular set are given in experiment 18 to 25 (pages 153 to 160). The results are summarised in table 3.6.

Table 3.6

Hydrolytic properties of 6-bromo saligenin cyclic phosphoramidothionates [pH 11.85, 0.0095M NaOH, Temp 30°C]



Alkylamide group	Code No.	in nm.	K_{hyd} (min^{-1})	Average K_{hyd} (min^{-1})	$t_{\frac{1}{2}}$ (hr.)
Dimethylamide	BR-6	294	1.52×10^{-4}		
		298	1.49×10^{-4}	1.49×10^{-4}	77.5
		300	1.47×10^{-4}		

Continue

Table 3.6 (Contd.)

Alkylamide group	Code No.	in nm.	K_{hyd} (min^{-1})	Average K_{hyd} (min^{-1})	$T_{\frac{1}{2}}$ (hr.)
Dipentylamide	BR-8	294	2.09×10^{-5}		
		298	1.97×10^{-5}	1.95×10^{-5}	591
		300	1.80×10^{-5}		
Dihexylamide	BR-9	294	1.09×10^{-5}		
		298	0.99×10^{-5}	1.01×10^{-5}	1148
		300	0.94×10^{-5}		
Dibutylamide	BR-10	294	1.91×10^{-5}		
		298	1.62×10^{-5}	1.72×10^{-5}	670
		300	1.63×10^{-5}		
2,6-Dimethylmorpholine	BR-12	294	2.79×10^{-5}		
		298	2.67×10^{-5}	2.69×10^{-5}	429
		300	2.61×10^{-5}		
2-Ethylpiperidine	BR-14	294	3.26×10^{-5}		
		298	3.29×10^{-5}	3.26×10^{-5}	354
		300	3.23×10^{-5}		
4-Benzylpiperidine	BR-15	294	0.74×10^{-5}		
		298	0.74×10^{-5}	0.71×10^{-5}	1677
		300	0.67×10^{-5}		
Hexamethyleneimide	BR-17	294	0.83×10^{-5}		
		298	0.78×10^{-5}	0.79×10^{-5}	1447
		300	0.76×10^{-5}		

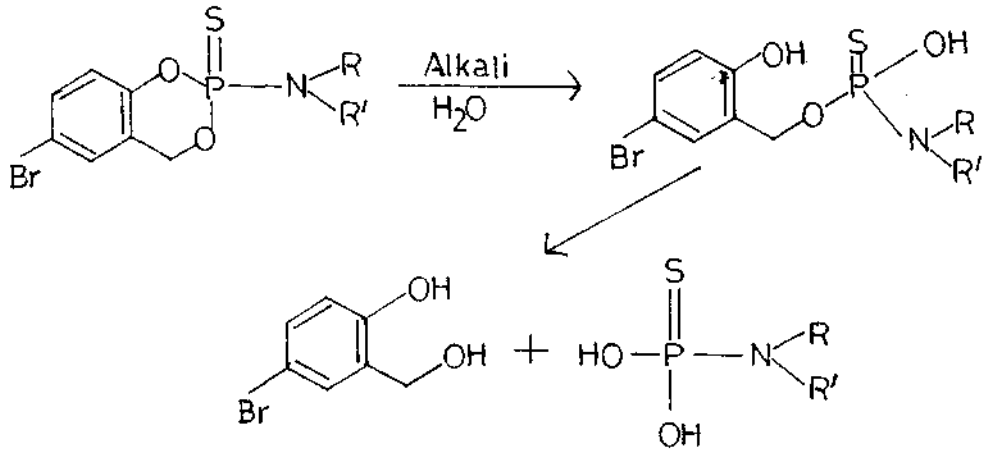
The average hydrolysis constants recorded in the table 3.6; show that the hydrolytic property of the compounds appears to relate with the size of the exocyclic substituent on the phosphorus atom. For 6-bromo saligenin cyclic phosphoramidothionates K_{hyd} increases in the order :

SR-15 < SR-7 < SR-9 < SR-10 < SR-8 < SR-12 < SR-14 < SR-6 i.e., the 4-benzylpiperidino compound (SR-15) is most stable, and the diisobutylamido compound (SR-6) is least stable to alkaline hydrolysis.

The dibutylamido compound (SR-10) is eight times more stable than the diisobutylamido compound (SR-6) to alkaline hydrolysis.

The 4-benzylpiperidino compound (SR-15) is 4.5 times more stable than 2-ethylpiperidino compound (SR-14) to alkaline hydrolysis.

The hydrolysis of the saligenin cyclic esters proceeds with the initial fission of the endo cyclic aryl ester bond, and the cleavage of the exocyclic ester bond does not take place by alkaline hydrolysis[28]. In our case we could not detect any freeamine in alkaline medium and there is no cleavage in the P-N bond. Following the work of Eto (Ref.28, p.74, equation 34) it may be proposed that the following reaction is taking place in course of alkaline hydrolysis.

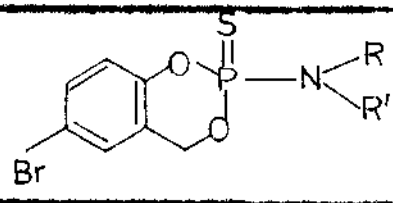


3.8.6 FUNGICIDAL ACTIVITY

The data (% inhibition) of some 4-bromo saligenin cyclic phospheramidothionates for *in vitro* growth inhibition studies against *Helminthosporium oryzae* (causal organism of brown spot disease of rice) are listed in experiments 26 to 33 (pages 161 to 168). The data for Hinosan (O-ethyl S,S, di-phenyl phosphorodithioate) have also been presented in experiment 34 (page 169). The ED_{50} and ED_{95} values ($\mu g/ml$) calculated by least square regression analysis are given in table 3.7 and 3.8.

Table 3.7

Antifungal activity of the phospheramidothionates against *H. oryzae* (Breda de Haan).



Alkylamide group	Code No.	ED_{50} in $\mu g/ml$			
		24 hrs	48 hrs	72 hrs	96 hrs
Diisobutylamide	BR-4	1.32	2.35	3.14	4.24
Dipentylamide	BR-8	0.67	1.51	2.27	3.03

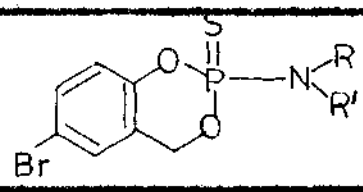
/Contd..

Table 3.7 (Contd.)

Alkylamide group	Code No.	ED_{50} in $\mu g/ml$			
		24 hrs	48 hrs	72 hrs	96 hrs
Dihexylamide	BR-9	1.33	2.89	3.38	4.03
Dibutylamide	BR-10	1.00	1.66	2.27	3.15
2,6-Dimethylmorpholine	BR-12	0.38	0.68	0.97	1.43
2-Ethylpiperidine	BR-14	0.95	1.43	1.95	2.36
4-Benzylpiperidine	BR-15	0.46	1.69	2.80	5.00
Hexamethyleneimide	BR-17	0.95	1.43	1.95	2.36
Hinosan (standard)	-	39.81	63.09	81.28	93.32

Table 3.8

Antifungal activity of the phosphoramidothionates against *H. oryzae* (Breda de Haan).



Alkylamide group	Code No.	ED_{95} in $\mu g/ml$			
		24 hrs	48 hrs	72 hrs	96 hrs
Diisobutylamide	BR-6	15.00	18.82	23.43	24.16
Dipentylamide	BR-8	8.63	20.18	24.50	27.06
Dihexylamide	BR-9	14.82	18.45	38.17	40.35
Dibutylamide	BR-10	10.13	18.90	23.99	51.37
2,6-Dimethylmorpholine	BR-12	3.98	4.30	6.14	8.02
2-Ethylpiperidine	BR-14	11.16	17.15	22.56	27.20

/Contd..

Table 3.8 (Contd.)

Alkylamide group	Code No.	ED_{95} in $\mu g/ml$			
		24 hrs	48 hrs	72 hrs	96 hrs
4-Benzylpiperidine	BR-15	3.30	26.04	33.30	60.66
Hexamethylenimide	BR-17	11.10	17.15	22.55	27.20
Hinosan (standard)	-	186.20	218.77	269.15	309.02

From the antifungal activity data it has been observed that the 6-bromo saligenin cyclic phosphoramidothionates show very good inhibitory effects on the growth of *H. oryzae*; their inhibitory effects are much greater than that of Hinosan.

The ED_{50} values for the 6-bromo saligenin cyclic phosphoramidothionates at 48 hrs increases in the following order :

BR-12 < BR-14 = BR-17 < BR-8 < BR-10 < BR-15 < BR-6 < BR-9 < Hinosan

i.e., the 2,6-dimethylmorpholine compound (BR-12) is most active and the dibenzylamide compound (BR-9) is least active. In case of BR-12 compound, the activity is found to be nearly 92 times greater than that of Hinosan; BR-9 compound is found to be nearly 24 times more active compared to Hinosan.

At 48 hrs the ED_{95} values of 6-bromo saligenin cyclic phosphoramidothionates increases in the following order :

BR-12 < BR-14 = BR-17 < BR-9 < BR-6 ≈ BR-10 < BR-8 < BR-15 < Hinosan

i.e., the 2,6-dimethylmorpholine compound (BR-12) is most active; and the 4-benzylpiperidine compound (BR-15) is least active. The activity of BR-12 compound is nearly 50 times greater than that of Hinosan, and the activity of BR-15 compound is nearly 8 times greater than that of Hinosan.

To sum up 2-(2,6-dimethylmorpholino)-6-bromo-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BR-12) is most effective against H. oryzae.

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

For structure activity correlation, regression yields several equation presented in table 3.9 for H. oryzae. The best fit Equation is Eq. 9.

$$pED_{50} = 25.622SIC - 27.694(SIC)^2 - 0.896 \log P + 0.486$$
$$(\pm 9.356) \quad (\pm 9.232) \quad (\pm 0.132) \quad (\pm 2.342)$$

where n= 13; s = 0.14; r = 0.94;

$$F_{(3,9)}\,cal = 23.9 \text{ and } F_{(3,9)(0.001)}\,tab = 13.9 \qquad \ldots\ldots (9)$$

In these equations, pED_{50} represents - $\log ED_{50}(M)$, n is the number of compounds, s is the standard deviation, r is the correlation co-

efficient and F is the statistical measure of the significance of correlation. The figures in parenthesis are the 99.9% confidence intervals of the corresponding constants. The conclusion is as follows :

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

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

(iii) The regression equation involving pED_{50} with SiC, $(SiC)^2$ and log P provides the best fit equation as judged by r, s and F values. Since log P is a hydrophobic parameter and SiC is a topological steric parameter, it suggests that the stereo-hydrophobic make-up and topology of the bioactive molecule are major determinants of the bioresponse.

Table 3.9

Regression Equations for [illegible]

	n	r	s	F_{cal}	F_{tab}
$pED_{50} = -3.716\ SIC + 6.931$ (± 1.517) (± 0.399)	13	0.79	0.30	1,11=6.00	(1,11)(0.05) = 4.80 ...(1)
$= 23.173\ SIC - 26.427(SIC)^2 + 0.200$ (± 19.583) (± 19.338) (± 4.047)	13	0.60	0.29	2,10=4.19	(2,10)(0.05) = 4.1 ...(2)
$= -2.328\ SIC - 0.887\ \log P + 7.481$ (± 0.065) (± 0.203) (± 0.502)	13	0.99	0.19	2,10=17.42	(2,10)(0.001) = 14.90 ...(3)
$= -2.450(SIC)^2 - 0.281(\log P)^2 + 6.249$ (± 1.018) (± 0.071) (± 0.269)	13	0.87	0.19	2,10=15.76	(2,10)(0.01) = 7.60 ....(4)
$= -10.231(SIC)^2 - 0.026(\pi)^2 + 6.228$ (± 3.730) (± 0.014) (± 1.245)	13	0.73	0.27	2,10=5.80	(2,10)(0.05) = 4.10 ....(5)
$= -9.394\ SIC - 0.182\pi + 10.260$ (± 5.099) (± 0.156) (± 3.212)	13	0.66	0.30	2,10=3.78	(2,10)(0.05) = 4.10 ....(6)
$= -2.751SIC - 3.468\ \log P + 0.473(\log P)^2 + 9.[illegible]$ (± 0.051) (± 1.642) ($\pm 0.[illegible]$) (± 1.376)	13	0.91	0.17	3,9=14.20	(3,9)(0.01) = 7.00 ....(7)
$-21.409\ SIC - 31.933(SIC)^2 - 0.228\pi + 3.411$ (± 18.312) (± 18.261) (± 0.145) (± 5.047)	13	0.76	0.27	3,9=4.03	(3,9)(0.05) = 3.90 ....(8)

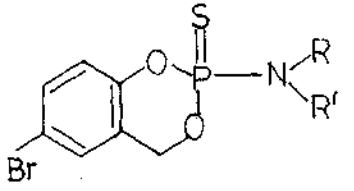
Table 3.9 (Contd.)

	n	r	s	F_{cal}	F_{tab}
$pED_{50} = 25.622SIC - 27.494(SIC)^2 - 0.896\log P + 0.486$ (±0.356) (±0.232) (±0.152) (±2.362)	13	0.94	0.14	3,9=23.90	(3,9)(0.001) =13.90 ..(9)
$= -0.227E_s + 0.127\pi - 0.014\pi^2 + 4.033$ (±0.093*) (±0.188) (±0.023) (±0.368)	13	0.75	0.27	3,9=3.85	(3,9)(0.05) =3.90 ...(10)
$= -0.175\ E_s - 0.113\log P - 0.217(\log P)^2 + 5.383$ (±0.070) (±1.974) (±0.041) (±1.594)	13	0.89	0.19	3,9=12.04	(3,9)(0.01) =9.00 ...(11)
$= -0.165\ E_s - 0.376\log P + 5.409$ (±0.061) (±0.208) (±0.415)	13	0.89	0.16	2,10 =19.74	(2,10)(0.001) = 14.90..(12)
$= -0.238\ E_s + 0.041\pi + 4.141$ (±0.091) (±0.045) (±0.261)	13	0.74	0.27	2,10=6.03	(2,10)(0.05) = 4.10 ..(13)
$= -2.338\log P + 0.449(\log P)^2 + 7.430$ (±2.172) (±0.722) (±1.395)	13	0.81	0.23	2,10=8.38	(2,10)(0.05) = 4.10 ..(14)

By using the best fit equation the value of the calculated and observed fungicidal activity of the compounds against *H. oryzae* and the value of the substituent constants are given in table 3.10.

Table 3.10

Value of substituent constants and calculated and observed fungicidal activity of the compounds against *M. arachn*.



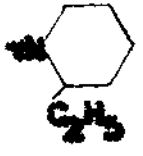
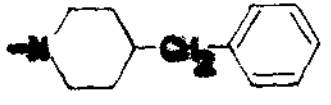
Compound Code No.	$-N\begin{matrix}R\\R^1\end{matrix}$	π	π^2	logP	$(\log P)^2$	SLC	$(SLC)^2$	E_s	pED_{50} (cal)	pED_{50} (obs)	ΔpED_{50}
BR-6	$-N(CH_2CH(CH_3)_2)_2$	4.76	22.65	1.60	2.56	0.47	0.22	-4.34	5.00	5.10	+0.10
BR-8	$-N((CH_2)_4CH_3)_2$	6.10	37.21	1.33	1.77	0.44	0.19	-3.28	5.31	5.27	-0.04
BR-9	$-N((CH_2)_5CH_3)_2$	7.18	51.55	1.36	1.85	0.41	0.17	-3.48	5.07	5.12	+0.05
BR-10	$-N((CH_2)_3CH_3)_2$	5.02	25.20	1.23	1.51	0.47	0.22	-3.08	5.33	5.24	-0.09
BR-12		2.05	4.20	0.98	0.96	0.50	0.30	-4.38	5.29	5.39	+0.10
BR-14		3.48	12.54	1.23	1.51	0.52	0.27	-3.41	5.23	5.28	+0.05
BR-15		5.29	33.52	1.12	1.25	0.49	0.24	-4.20	5.39	5.19	-0.20

Table 3.10 (contd.)

Compound Code No.	$-N<^{R}_{R^1}$	π	π^2	logP	$(\log P)^2$	SiC	$(SiC)^2$	E_s	pED_{50} (cal)	pED_{50} (obs)	ΔpED_{50}
SR-17		3.87	14.96	1.36	1.89	0.52	0.27	-2.03	5.20	5.27	+0.07
*SR-1		2.55	6.50	2.01	4.04	0.38	0.33	-0.86	4.41	4.36	-0.05
*SR-2		3.21	10.30	1.54	2.34	0.35	0.30	-2.10	4.89	4.74	-0.15
*SR-3		0.73	0.58	1.32	2.31	0.39	0.35	-2.10	4.55	4.63	+0.08
*SR-4	$-N<^{CH_3}_{CH_3}$	1.78	3.17	1.39	1.93	0.39	0.35	-1.48	4.67	4.53	-0.14
*SR-5	$-N<^{C_2H_5}_{C_2H_5}$	2.86	8.18	1.79	3.20	0.54	0.29	-2.42	4.69	4.81	+0.12

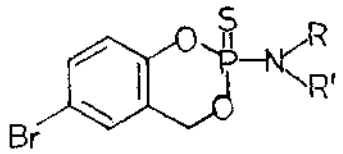
*SR-1 to SR-5 compound prepared in our laboratory by Das et al.[20] have been taken for regression analysis.

3.9 FURTHER STUDIES ON ANTIFUNGAL ACTIVITY OF SOME BROMO SALIGENIN CYCLIC PHOSPHORAMIDOTHIONATES AGAINST PYRICULARIA ORYZAE

Antifungal activities of some bromo saligenin cyclic phosphoramidothionates against *Pyricularia oryzae* (causal organism of blast disease of rice) have been studied according to the method described in section 3.6. Linear growth of the fungal discs were measured after 48, 72, 96 and 120 hours interval. The data (% inhibition) are listed in experiments 35 to 42 (pages 170 to 177). The ED_{50} and ED_{95} values ($\mu g/ml$) calculated by least square regression analysis are given in table 3.11 and 3.12.

Table 3.11

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

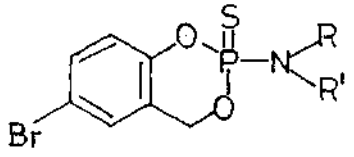


—N<R, R¹>	Compound Code No.	ED_{50} in µg/ml			
		48 hrs	72 hrs	96 hrs	120 hrs
Diisobutylamido	BP-6	0.30	0.41	0.58	0.82
Dipentylamido	BP-8	0.40	0.61	0.86	1.40
Dihexylamido	BP-9	0.45	0.61	0.93	1.66
Dibutylamido	BP-10	0.66	1.21	1.59	2.58
2,6-Dimethylmorpholino	BP-12	0.16	0.44	0.74	0.93
2-Ethylpiperidino	BP-14	0.54	0.94	1.36	2.12
4-Benzylpiperidino	BP-15	0.86	1.45	2.22	2.97
Hexamethylenimido	BP-17	0.54	1.73	2.21	3.49

Table 3.12

Antifungal activity of the phosphoramidothionates against

A. niger (aav)



– N R, R'	Compound Code No.	EC_{95} in μg/ml			
		48 hrs	72 hrs	96 hrs	120 hrs
Diisobutylamide	BR-6	1.51	2.31	4.11	6.23
Dipentylamide	BR-8	3.08	3.85	6.00	10.85
Dihexylamide	BR-9	2.64	4.83	8.14	14.53
Dibutylamide	BR-10	3.72	7.70	9.85	21.04
2,6-Dimethylmorpholine	BR-12	1.41	1.61	3.20	4.14
2-Ethylpiperidine	BR-14	4.46	6.95	10.30	17.31
4-Benzylpiperidine	BR-15	4.48	6.48	11.70	16.72
Hexamethylenimide	BR-17	5.76	9.86	14.51	23.69

From the antifungal activity data it has been observed that the 6-bromo saligenin cyclic phosphoramidothionates show good inhibitory effect on the growth of *K. oxysporum*.

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

BR-12 ≈ BR-6 < BR-8 = BR-9 < BR-14 < BR-10 < BR-15 < BR-17

i.e., the 2,6-dimethylmorpholino compound (BR-12) and diisobutylamide compound (BR-6) are most active and hexamethyleneimide compound (BR-17) is least active.

At 72 hrs the ED_{95} value of the 6-bromo saligenin cyclic phosphoramidothionates increases in the following order :

BR-12 < BR-6 < BR-8 < BR-9 < BR-15 < BR-14 < BR-10 < BR-17

i.e., the 2,6-dimethylmorpholino compound (BR-12) is most active and hexamethyleneimide compound (BR-17) is least active.

To sum up, the 2-(2,6-dimethylmorpholino-6-bromo)-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BR-12) is most effective and hexamethyleneimide-6-bromo-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BR-17) is least effective against *K. oxysporum*.

CHAPTER 9

STUDIES ON SOME 9-OXO/OHO/OAc SALIGENIN CYCLIC PHOSPHORAMIDO/THIONAMIDE INSECT PESTICIDAL ACTIVATORS

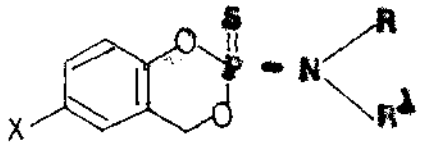
Chapter - 4

STUDIES ON SOME 6-NITRO/CHLORO SALIGENIN CYCLIC PHOSPHORAMIDOTHIONATES HAVING PESTICIDAL ACTIVITIES

In our laboratory some 6-nitro/chloro saligenin cyclic phosphoramidothionates have been prepared and structure of these compounds have been elucidated by Das *et al* (unpublished). I have studied some biological properties and chemical hydrolysis of these compounds, such as oral insecticidal activity on cockroaches (*P. americana*), contact insecticidal activity on blow-flies (*Chrysomya megacephala*), acute oral toxicity on rats, anticholinesterase activity in goat whole blood and blow-fly head homogenate, fungicidal activity on *Helminthosporium oryzae* and *Pyricularia oryzae* and chemical hydrolysis in 0.0095(M) NaOH in 50% ethanol (pH 11.85) at 30°C.

The physical properties of some 6-nitro/chloro saligenin cyclic phosphoramidothionates are given in table 4.1.

Table 4.1

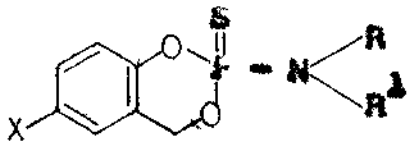


Compound Code No.	X	-N<R, R¹>	Yield(%)	Mol.wt.	M.P.(°C)
BD-25	NO_2	Diisobutylamide	80	358.00	136
BD-29	NO_2	Dibutylamide	80	358.00	101
BD-31	NO_2	2,6-Dimethyl-morpholine	90	344.00	145
CL-6	Cl	Diisobutylamide	78	347.70	91
CL-10	Cl	Dibutylamide	75	347.70	liquid
CL-12	Cl	2,6-Dimethyl-morpholine	71	333.45	94

ORAL INSECTICIDAL ACTIVITY ON COCKROACH

The insecticidal activity tests were performed on the American cockroaches, *P. americana* (Linn) according to the method described in section 3.1 and the results of the insecticidal activity have been listed in table 4.2.

Table 4.2



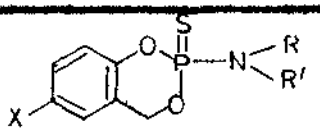
Compound Code No.	X	$-N<^{R}_{R^1}$	Conc. showing 100% mortality (LC_{100} µg/g)
BD-25	NO_2	Diisobutylamide	>75
BD-29	NO_2	Dibutylamide	>75
BD-31	NO_2	2,6-Dimethyl-morpholine	>75
Cl-6	Cl	Diisobutylamide	>75
Cl-10	Cl	Dibutylamide	>75
Cl-12	Cl	2,6-Dimethylmorpholine	10

The data presented in table 4.2 reveal that all phosphoramidothionates have less oral insecticidal activity on cockroaches (*P. americana*) except Cl-12 which has equal insecticidal activity compared with malathion.

CONTACT INSECTICIDAL ACTIVITY ON BLOW-FLY

Contact insecticidal activity tests on blow-flies, *Chrysomya megacephala* were performed according to Eto *et al* (30). Acetone solution of the individual compounds was topically applied to female adult insects. To 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 24h of the experiment. The results of the insecticidal activity have been listed in table 4.3.

Table 4.3



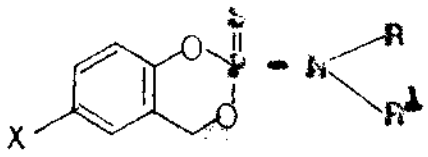
Compound Code No.	X	$-N\begin{matrix}R\\R^1\end{matrix}$	LD_{50} µg/female blow-fly
BD-25	NO_2	Diisobutylamide	>10
BD-29	NO_2	Dibutylamide	>10
BD-31	NO_2	2,6-Dimethylmorpholine	>10
Cl-6	Cl	Diisobutylamide	>10
Cl-10	Cl	Dibutylamide	>10
Cl-12	Cl	2,6-Dimethylmorpholine	10
BR-6	Br	Diisobutylamide	>10
BR-8	Br	Dipentylamide	>10
BR-9	Br	Dihexylamide	>10
BR-10	Br	Dibutylamide	>10
BR-12	Br	2,6-Dimethylmorpholine	>10
BR-14	Br	2-Ethylpiperidine	>10
BR-15	Br	4-Benzylpiperidine	>10
BR-17	Br	Hexamethylenimide	>10

The data presented in table 4.3 reveal that all phosphoramidothionates have less insecticidal activity on blow-flies (*Chrysomya megacephala*) except Cl-12.

ACUTE ORAL TOXICITY ON RATS

The acute oral toxicity on male white albino rats was performed according to the method described in section 3.2 and the results have been listed in table 4.4.

Table 4.4



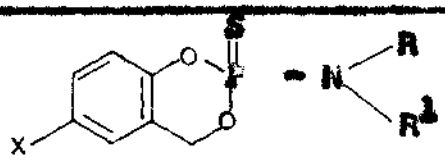
Compound Code No.	X	$-N\begin{matrix}R\\R^1\end{matrix}$	LD_{50} (mg/kg) male rat
BD-85	NH_2	Diisobutylamido	>250
BD-89	NH_2	Dibutylamido	>250
BD-91	NH_2	2,6-Dimethylmorpholino	>250
Cl-6	Cl	Diisobutylamido	>250
Cl-10	Cl	Dibutylamido	>250
Cl-12	Cl	2,6-Dimethylmorpholino	>250

The data show that all phosphoramidothionates have less acute oral toxicity on rats.

ANTICHOLINESTERASE ACTIVITY

The anticholinesterase activity of some phosphoramidothionates in blow-fly head homogenate and goat whole blood has been measured by the method described in section 3.3 and the results have been summarised in table 4.5.

Table 4.5



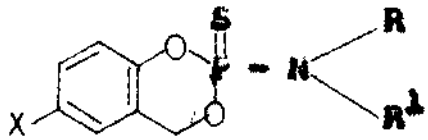
Compound Code No.	X	$-N\begin{matrix}R\\R^1\end{matrix}$	I_{50}(M) (BFAChE) blow-fly	I_{50}(M) (AChE) goat-whole blood
BD-25	NO_2	Diisobutylamide	2.76×10^{-5}	7.20×10^{-5}
BD-29	NO_2	Dibutylamide	9.48×10^{-5}	4.16×10^{-5}
BD-31	NO_2	2,6-Dimethylmorpholino	1.38×10^{-5}	0.16×10^{-5}
Cl-6	Cl	Diisobutylamide	80.37×10^{-8}	4.25×10^{-8}
Cl-10	Cl	Dibutylamide	9.49×10^{-8}	4.13×10^{-8}
Cl-12	Cl	2,6-Dimethylmorpholino	0.20×10^{-8}	2.07×10^{-8}

The data presented in table 4.5 reveal that nitro compounds are more inhibited than chloro compounds.

FUNGICIDAL ACTIVITY

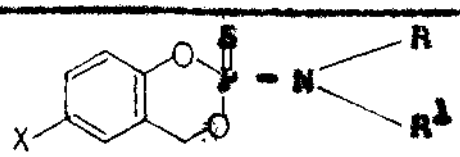
Antifungal activities of these compounds against *Helminthosporium oryzae* and *Pyricularia oryzae* have been studied according to the method described in section 3.6. The ED_{50}(µg/ml) and ED_{95}(µg/ml) values of fungicidal activity at 72h have been summarized in table 4.6 and table 4.7 for *H. oryzae* and *P. oryzae* respectively.

Table 4.6 (for *H. oryzae*)



Compound Code No.	X	-N(R)(R¹)	ED_{50}(µg/ml) at 72 hrs	ED_{95}(µg/ml) at 72 hrs
BD-25	NO_2	Diisobutylamide	237.23	546.35
BD-29	NO_2	Dibutylamide	137.37	712.17
BD-31	NO_2	2,6-Dimethylmorpholine	100.20	442.30
Cl-6	Cl	Diisobutylamide	2.72	18.96
Cl-10	Cl	Dibutylamide	2.03	20.24
Cl-12	Cl	2,6-Dimethylmorpholine	0.80	4.90
BR-6	Br	Diisobutylamide	3.14	23.43
BR-10	Br	Dibutylamide	2.27	23.99
BR-12	Br	2,6-Dimethylmorpholine	0.97	6.14

Table 4.7 (for *P. oryzae*)



Compound Code No.	X		ED_{50} (µg/ml) at 72 hrs	ED_{95} (µg/ml) at 72 hrs.
BD-25	NO_2	Diisobutylamide	12.60	85.94
BD-29	NO_2	Dibutylamide	12.65	78.24
BD-31	NO_2	2,6-Dimethyl-morpholine	9.95	63.49
Cl-6	Cl	Diisobutylamide	0.84	4.70
Cl-10	Cl	Dibutylamide	0.58	3.99
Cl-12	Cl	2,6-Dimethyl-morpholine	0.38	2.63
BR-6	Br	Diisobutylamide	0.41	2.31
BR-10	Br	Dibutylamide	1.21	7.70
BR-12	Br	2,6-Dimethyl-morpholine	0.44	1.61

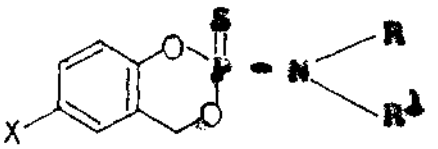
From the tables 4.6 and 4.7 it has been observed that the inhibitory effects of 6-chloro/bromo saligenin cyclic phosphoramidothionates on the growth of *M. grisea* and *P. oryzae* are greater than that of corresponding 6-nitro saligenin cyclic phosphoramidothionates.

Hence it may be assumed that the antifungal activity of the phosphoramidothionates against *H. sativa* and *K. sativa* is significantly influenced by the nature of the substituent present at the 6-position of the saligenin cyclic phosphoramidothionates.

CHEMICAL HYDROLYSIS

The chemical hydrolysis in 0.0992(M) NaOH solution in 50 percent ethanol (pH 11.85) of these compounds were studied according to the method described in section 3.5. The results have been listed in table 4.8.

Table 4.8

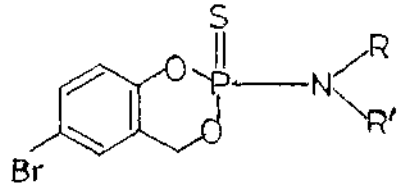


Compound Code No.	X	-N<R, R¹>	Average K_{hyd} (min^{-1})
SD-28	NO_2	Diisobutylamide	3.54×10^{-5}
SD-29	NO_2	Dibutylamide	3.80×10^{-5}
SD-31	NO_2	2,6-Dimethylmorpholine	1.16×10^{-5}
Cl-6	Cl	Diisobutylamide	0.58×10^{-5}
Cl-10	Cl	Dibutylamide	0.89×10^{-5}
Cl-12	Cl	2,6-Dimethylmorpholine	4.06×10^{-5}

The average hydrolysis constants recorded in table 4.8 show that nitro/chloro saligenin cyclic phosphoramidothionates are stable towards alkaline hydrolysis (pH 11.85).

GENERAL CONCLUSIONS AND REMARKS

(i) The following 2-alkylamido-6-bromo-4H-1,3,2-benzodioxaphosphorin 2-sulphides have been synthesized and examined for



where $-N\begin{matrix}R\\R^1\end{matrix}$ = Diisobutylamide
= Dipentylamide
= Dihexylamide
= Dibutylamide
= 2,6-Dimethylmorpholine
= 2-Ethylpiperidine
= 4-Benzylpiperidine
= Hexamethyleneimide

oral insecticidal activity on cockroach (P. americana), acute oral toxicity on rats, anticholinesterase activity in goat whole blood and in blow-fly head homogenate, phytotoxicity on rice seed (Pusa 2-21 variety), chemical hydrolysis in 0.0000(M)NaOH in 50 percent ethanol (pH 11.83) and fungicidal activity on Helminthosporium oryzae.

The 6-bromo saligenin cyclic phosphoramidothionates have been prepared by the reaction of the appropriate alkylamidophosphorodichloridothionate with 5-bromo saligenin at low temperature (0-8°C) in presence of K_2CO_3 as dehydrogen chloride agent.

(ii) All the compounds show common IR bands: 1010-1040 cm^{-1}(s), P-O-C (Alkyl), 1235-1240 cm^{-1}(s) and 870-910 cm^{-1}(s), P-O-C(aryl); 805-815 cm^{-1}, P=S(I); 640-690 cm^{-1}, P=S(II).

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

(iv) For the proton noise decoupled ^{13}C spectra of BR-6, BR-12, BR-14 and BR-17 at 100 MHz the saturated carbons 2 and 3 bands from the phosphorus show splitting due to ^{13}C - ^{31}P spin coupling.

The chemical shift values for the CH_2 carbon (C_4) in the dioxaphosphorin ring of BR-6, BR-12, BR-14 and BR-17 are almost the same. ^{13}C chemical shifts are often quite sensitive to conformation, it supports the similarity of the structure.

The ^1H NMR spectra of the o-bromo caligenin cyclic phosphoramidothionates have signals at δ = 4.65-4.74 ppm for the $-CH_2-$ group protons in the dioxaphosphorin ring and for all the compounds the signal is a eight line multiplet.

From the ^1H NMR spectral studies of the 2-alkoxy/phenoxy/alkylamido-4-nitro-4H-1,3,2-benzodioxaphosphorin 2-sulphides it is fairly evident that the chemical shift difference of the two geminal protons H_{4A} and H_{4B} (two protons of the CH_2 group in the dioxaphosphorin ring) increases in going from the methoxy (BD-8) to the cyclohexylamido (BD-10) compound. Also the 2-substituent

is at the same time increases in bulk, and probably spends more time in the conformation with least steric interactions. In BD-10, this may be mainly in one conformation (conformation II, P.73), while in BD-8 it appears that both the chemical shift difference and the difference between $^3J_{P-O-C-H_{4A}}$ and $^3J_{P-O-C-H_{4B}}$ are quite small. This suggests that the molecule exists as an average of the two conformations with rapid interconversion between them. In case of BD-9, it seems that an intermediate situation prevails. In case of BD-10, several plot expansions and decoupling experiments suggest that the quasi-equatorial proton H_{4B} is assigned to 5.2 ppm proton (J = 23.0 Hz) and the quasi-axial proton H_{4A} is assigned to the 5.6 ppm (J = 6.3 Hz); the geminal coupling constant is 14.7 Hz. It has also been observed that the proton H_{4B} is coupled equally to the three aromatic protons H_5, H_7 and H_8 with J = 0.6 Hz; the proton H_{4A} is more strongly coupled to H_5 and H_7 with J = 1.0 Hz, but not to H_8.

(v) o-bromo saligenin cyclic phosphoramidothionates are almost non insecticidal to cockroaches.

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

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

(viii) None of the compounds is phytotoxic to rice seed (Pusa 2-21 variety) at 100 ppm and 50 ppm. At 200 ppm the compounds show slight phytotoxicity except 2,6-dimethylmorpholine compound.

(ix) From the chemical hydrolysis it has been observed that 6-bromo saligenin cyclic phosphoramidothionates are most stable to alkaline hydrolysis (pH 11.89).

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

(xi) Quantitative Structure Activity Relationship show that a good correlation is obtained between the pED_{50} value (for *H. oryzae* at 72 hrs) and Structural Information Content (SIC) and hydrophobic parameter (log P). The equation is

$$pED_{50} = \underset{(\pm 0.356)}{23.622SIC} - \underset{(\pm 0.232)}{27.494(SIC)^2} - \underset{(\pm 0.132)}{0.896 \log P} + \underset{(\pm 2.362)}{0.486}$$

$n = 13$; $s = 0.14$; $r = 0.94$

$F_{(3,9)}cal = 23.9$ and $F_{(3,9)(0.001)}tab = 12.9$

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

(xii) The antifungal studies (by growth inhibition) against *F. oryzae* indicate that 6-bromo saligenin cyclic phosphoramidothionates show very good inhibitory effect on the growth of

P. oryzae. Of all the compounds 2-(2,6-Dimethylmorpholino)-6-bromo-4H-1,3,2-benzodioxaphosphorin 2-sulphide is most effective against *P. oryzae*.

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

(a) all the compounds have less insecticidal activity on cockroaches, except Cl-12 which has equal insecticidal activity compared with salithion.

(b) all the compounds have less contact insecticidal activity on blow-flies, except Cl-12.

(c) all the compounds have less acute oral toxicity on rats.

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

(e) the 6-chloro/bromo saligenin cyclic phosphoramidothionates have good inhibitory effects on the growth of *H. oryzae* and *P. oryzae* compared with that of 6-nitro saligenin cyclic phosphoramidothionates.

Hence it may be assumed that the antifungal activity of the phosphoramidothionates against *H. oryzae* and *P. oryzae* is significantly influenced by the nature of the substituent present at the 6-position of the saligenin cyclic phosphoramidothionates.

(f) all the compounds are stable towards alkaline hydrolysis at pH 11.50.

(xiv) The biological activities and other data justify further examination of these compounds as potential fungicides. Whether the use of these cyclic phosphorus compounds will protect the plants from diseases in the field remains to be studied. In order to find out the chemical structure - biological activity relationship in these 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.

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EXPERIMENTS

Experiment - 1

Antichelinesterase inhibition in *Chrysomya megacephala* (Blow-fly) head homogenate (BFAChE) at 30°C of SR-6
(Phosphate buffer, pH 8.0; total volume = 3.15 ml/1 fly head, λ = 625 nm; incubation time 30 min.)

Sol.	Inhibitor* Conc. (μg)	O.D.	ΔO.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	0.059	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 = 14.86
r = 0.97

Experiment 2

Acetylcholinesterase inhibition in *Chrysomya megacephala* (Blow-fly) head homogenate (BFAChE) at 30°C of BH-6

(Phosphate buffer, pH 8.0; total volume = 3.15 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	-	0.416	-	-	
1	16	0.394	0.022	5.28	
2	30	0.382	0.034	8.17	
3	45	0.374	0.042	10.09	2.35×10^{-8}
4	60	0.357	0.059	14.18	
5	75	0.348	0.068	16.34	
6	90	0.342	0.074	17.78	

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

Regression constants : Y = mX + C

C = -[illegible]

m = [illegible]

r = [illegible]

Experiment 3

Acetylcholinesterase inhibition in *Curvimaya nonnenchala* (Blow-fly) head homogenate (BFAChE) at 30°C of ER-9

(Phosphate buffer, pH 8.0; total volume = 3.15 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	-	0.416	-	-	
1	15	0.403	0.013	3.13	
2	30	0.380	0.036	8.65	
3	45	0.372	0.044	10.57	2.00×10^{-8}
4	60	0.359	0.057	13.70	
5	75	0.347	0.069	16.58	
6	90	0.336	0.080	19.23	

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

Regression constants : Y = mX + C

C = -39.22

m = 18.91

r = 0.98

Experiment 4

Acetylcholinesterase inhibition in *Curvicauda mesocephala* (Blow-fly) head homogenate (BF/ChE) at 30°C of BH-19
(Phosphate buffer, pH 8.0; total volume = 5.15 ml/1 fly head, λ = 425 nm; incubation time 30 min.)

Sets	Inhibitor* Conc. (μg)	O.D.	ΔO.D.	% Inhibition (Y)	I_{50}(μ)
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^{-2}
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 = 17.03

r = 0.95

Experiment 5

Acetylcholinesterase inhibition in *Chrysomya megacephala* (Blow-fly) head homogenate (BFAChE) at 30°C of BR-12

(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	-	0.416	-	-	
1	16	0.410	0.007	1.68	
2	30	0.393	0.023	5.52	
3	50	0.373	0.043	10.33	5.14×10^{-3}
4	60	0.370	0.046	11.05	
5	75	0.366	0.050	12.01	
6	90	0.352	0.064	15.38	

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

Regression constants : Y = mX + C

C = -19.66

m = 17.42

r = 0.998

Experiment 6

Acetylcholinesterase inhibition in *Curvicavia macrocephala* (Blow-fly) head homogenate (BFACH) at 30°C or RH = 14

(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	[illegible]	-	
1	15	0.366	0.032	8.04	
2	30	0.358	0.040	10.05	
3	45	0.348	0.050	12.56	1.97×10^{-5}
4	60	0.336	0.062	15.57	
5	75	0.320	0.078	19.59	
6	90	0.308	0.090	22.61	

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

Regression constants : $Y = mX + C$

$C = -15.48$

$m = 18.29$

$r = 0.936$

Experiment 7

Acetylcholinesterase inhibition in Chrysomya megacephala (Blow-fly) head homogenate (BF/ChE) at 30°C of BR - 13

(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	-	0.376		-	
1	15	0.356	0.020	5.32	
2	30	0.352	0.024	6.38	
3	45	0.349	0.027	7.18	4.47×10^{-8}
4	60	0.335	0.041	10.90	
5	75	0.321	0.055	14.62	
6	90	0.302	0.074	19.68	

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

Regression constants : Y = mX + C

C = -16.95

m = 16.73

r = 0.86

Experiment 8

Acetylcholinesterase inhibition in Chrysomya megacephala (Blow-fly) head homogenate (BF/ChE) at 30°C of BR - 17

(Phosphate buffer, pH 8.0; total volume = 3.15 ml/1 fly head, λ=625 nm; incubation time 30 min.)

Sets	Inhibitor Conc.(μg)	O.D.	Δ O.D.	% Inhibition (Y)	$I_{50}(\mu)$
Control	-	0.416	-	-	
1	15	0.395	0.021	5.04	
2	30	0.380	0.036	8.65	
3	45	0.370	0.046	11.05	2.40×10^{-3}
4	60	0.359	0.057	13.70	
5	75	0.347	0.069	16.58	
6	90	0.330	0.086	20.67	

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

Regression constants : Y = mX + C
C = -18.22
m = 18.72
r = 0.96

Experiment - 9

Acetylcholinesterase inhibition in goat whole blood of BR - 6 at 30°C

(Phosphate buffer, pH 8.0; total volume = 3.15 ml/0.2 ml blood plasma;

λ = 625 nm; incubation time 30 min.)

Sets	Inhibitor Conc. (μg)	O.D.	ΔO.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	
4	90	0.913	0.102	10.04	28.93×10^{-3}
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 = 13.86

r = 0.975

Experiment 10

Acetylcholinesterase inhibition in goat whole blood at 30°C of BR - 8

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

Sets	Inhibitor* Conc. (μg)	O.D.	ΔO.D.	% Inhibition (Y)	I_{50}(M)
Control	-	1.072	-	-	
1	15	0.980	0.092	8.58	
2	30	0.943	0.129	12.03	
3	60	0.925	0.147	13.71	
4	90	0.900	0.172	16.04	14.03×10^{-9}
5	120	0.868	0.204	19.02	
6	150	0.825	0.247	23.04	

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

Regression constants : Y = mX + C

C = -7.25

m = 12.78

r = 0.95

Experiment 11

Acetylcholinesterase inhibition in goat whole blood at 30°C of BR - 9 (Phosphate buffer, pH 8.0; total volume = 3.13 ml/0.2 ml blood plasma; λ = 625 nm; incubation time 30 min.)

Sets	Inhibitor* Conc. (μg)	O.D.	Δ O.D.	% Inhibition (Y)	$I_{50}(\mu)$
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	
4	90	0.873	0.199	18.56	1.15×10^{-3}
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 : $Y = mX + C$

$C = -17.20$

$m = 19.80$

$r = 0.89$

Experiment 12

Acetylcholinesterase inhibition in goat whole blood at 30°C of BR - 10

(Phosphate buffer, pH 8.0; total volume = 3.13 ml/0.2 ml blood plasma; λ= 625 nm; incubation time 30min.)

Sets	Inhibitor* Conc. (μg)	O.D.	Δ 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 constants : Y = mX + C

C = -23.31

m = 21.42

r = 0.946

Experiment 13

Acetylcholinesterase inhibition in goat whole blood at 30°C of BR - 12

(Phosphate buffer, pH 8.0; total volume = 3.15 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	-	1.015	-	-	
1	15	0.971	0.044	4.33	
2	30	0.954	0.061	6.00	
3	60	0.928	0.087	8.57	28.24×10^{-8}
4	90	0.906	0.109	10.73	
5	120	0.862	0.153	15.07	
6	150	0.831	0.184	18.12	

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

Regression constants : Y = mX + C

C = -13.26

m = 12.35

r = 0.94

Experiment 14

Acetylcholinesterase inhibition in goat whole blood plasma at 30°C of BR - 14

(Phosphate buffer, pH 8.3; total volume = 3.13 ml/0.2 ml blood plasma; λ = 625 nm; incubation time 30 min.)

Sets	Inhibitor* Conc. (µg)	O.D.	ΔO.D.	% inhibition (Y)	I_{50}(M)
Control	-	1.015	-	-	
1	15	0.972	0.043	4.23	
2	30	0.923	0.092	9.06	
3	60	0.885	0.130	12.80	0.70×10^{-3}
4	90	0.837	0.178	17.53	
5	120	0.762	0.253	24.92	
6	150	0.710	0.305	30.04	

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

Regression constants : Y = mX + C
C = -27.73
m = 24.84
r = 0.95

Experiment 15

Acetylcholinesterase inhibition in goat whole blood at 30°C of BR - 15

(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.	ΔO.D.	% Inhibition (Y)	I_{50}(M)
Control	-	1.015	-	-	
1	15	0.986	0.029	2.85	
2	30	0.963	0.052	5.12	
3	60	0.937	0.078	7.68	
4	90	0.909	0.106	10.44	32.80×10^{-6}
5	120	0.887	0.128	12.61	
6	150	0.837	0.178	17.53	

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

Regression constants : $Y = mX + C$

$C = -13.09$

$m = 13.12$

$r = 0.94$

Experiment 16

Acetylcholinesterase inhibition min goat whole blood at 30°C of BR - IV

(Phosphate buffer, pH 8.0; total volume = 3.15 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 17

Molar extinction co-efficients of the compounds at different wavelengths in 0.0095(M) NaOH (in 30% ethanol) Soln. pH 11.85

Compound	Molar extinction co-efficient		
	294 nm	299 nm	300 nm
Brome saligenin	2646	3039	3132
BR-6	402.4	503	503
BR-8	862.8	615.84	619.46
BR-9	397.86	393.32	267.46
BR-10	287.5	299.6	272.4
BR-12	264.7	230.71	226.9
BR-14	930.4	831.6	910.8
BR-15	1291.8	1199.5	1133.4
BR-17	236.6	170.4	149

Experiment 18

Chemical hydrolysis of Compound BR-4

pH 11.85, 0.0993(M) NaOH (in 50% ethanol)

Initial concentration $C_o = 0.994 \times 10^{-4}$(M), Temperature 30°C

λ (nm)	Time (hours)	O.D.	C_t(M)	$\log \frac{C_o}{C_o - C_t}$	K_{hyd} (min^{-1})	Average K_{hyd} (min^{-1})	$T_{\frac{1}{2}}$(hr.)
294	0	0.040	-	-	1.32×10^{-4}		
	240	0.238	0.852×10^{-4}	0.946			
296	0	0.050	-	-	1.49×10^{-4}	1.49×10^{-4}	77.5
	240	0.270	0.877×10^{-4}	0.929			
300	0	0.050	-	-	1.47×10^{-4}		
	240	0.280	0.874×10^{-4}	0.918			

Experiment 19

Chemical hydrolysis of Compound ER-8

pH 11.85, 0.0095(M) NaOH (in 50% ethanol)

Initial concentration $C_o = 4.320 \times 10^{-4}$(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})	$T_{\frac{1}{2}}$(hr.)
294	0	0.39	-	-	2.09×10^{-5}		
	240	0.60	1.177×10^{-4}	0.131			
298	0	0.31	-	-	1.97×10^{-5}		
	240	0.57	1.119×10^{-4}	0.122			
300	0	0.28	-	-	1.80×10^{-5}	1.95×10^{-5}	591
	240	0.54	1.034×10^{-4}	0.113			

Experiment 20

Chemical hydrolysis of Compound BR-9

pH 11.83, 0.0395(M) NaOH (in 50% ethanol)

Initial concentration, $C_0 = 6.35 \times 10^{-4}$(M), Temperature 30°C

λ (nm)	Time (hour)	O.D.	C_t(M)	$\log \frac{C_0}{C_0 - C_t}$	K_{hyd} (min^{-1})	Average K_{hyd} (min^{-1})	$T_{\frac{1}{2}}$(hr.)
294	0	0.38	-	-	1.09×10^{-5}		
	240	0.37	0.827×10^{-4}	0.008			
296	0	0.25	-	-	0.99×10^{-5}	1.01×10^{-5}	1148
	240	0.472	0.848×10^{-4}	0.062			
300	0	0.17	-	-	0.84×10^{-5}		
	240	0.401	0.806×10^{-4}	0.039			

Experiment 21

Chemical hydrolysis of Compound ER-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})	$T_{\frac{1}{2}}$(hr.)
294	0	0.19	-	-	1.91×10^{-3}		
	240	0.38	0.892×10^{-4}	0.120			
298	0	0.11	-	-			
	240	0.319	0.771×10^{-4}	0.102	1.63×10^{-3}	1.72×10^{-3}	670
300	0	0.101	-	-	1.63×10^{-3}		
	240	0.322	0.772×10^{-4}	0.102			

Experiment 22

Chemical hydrolysis of Compound BR-12

pH 11.85, 0.0092(M) NaOH (in 50% ethanol)

Initial concentration, $C_0 = 2.644 \times 10^{-4}$(M), Temperature 30°C.

λ (nm)	Time (hour)	O.D.	C_t(M)	$\log \frac{C_0}{C_0 - C_t}$	K_{hyd} (min^{-1})	Average K_{hyd}(min^{-1})	$T_{\frac{1}{2}}$(hrs)
294	0	0.07	-	-	2.79×10^{-5}		
	240	0.279	0.877×10^{-4}	0.175			
296	0	0.061	-	-	2.67×10^{-5}	2.69×10^{-5}	429
	240	0.296	0.845×10^{-4}	0.167			
300	0	0.06	-	-	2.61×10^{-5}		
	240	0.331	0.829×10^{-4}	0.163			

Experiment - 23

Chemical hydrolysis of Compound SH-14

pH 11.85, 0.0095(M) NaOH (in 30% ethanol)

Initial concentration, $C_o = 2.223 \times 10^{-4}$(M), Temperature 30°C

λ (nm)	Time (hour)	O.D.	C_t(M)	$\log \frac{C_o}{C_o - C_t}$	K_{hyd}	Average K_{hyd}(min^{-1})	$\tau_{\frac{1}{2}}$(hr.)
294	0	0.24	-	-	3.26×10^{-5}		
	240	0.401	0.849×10^{-4}	0.204			
296	0	0.21	-	-	3.29×10^{-5}	3.26×10^{-5}	354
	240	0.418	0.935×10^{-4}	0.206			
300	0	0.23	-	-	3.23×10^{-5}		
	240	0.439	0.840×10^{-4}	0.202			

Experiment - 24

Chemical Hydrolysis of Compound BP-19

pH 11.83, 0.0093(M) NaOH (in 30% ethanol)

Initial concentration $C_o = 4.335 \times 10^{-4}$(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})	$T_{\frac{1}{2}}$(hr.)
294	0	0.56	-	-	0.74×10^{-3}		
	240	0.62	0.44×10^{-4}	0.346			
298	0	0.52	-	-	0.74×10^{-3}		
	240	0.60	0.44×10^{-4}	0.346		0.71×10^{-3}	16.17
300	0	0.50	-	-	0.67×10^{-3}		
	240	0.58	0.40×10^{-4}	0.342			

Experiment - 23

Chemical hydrolysis of Compound SR-17

pH 11.83, 0.0095(M) NaOH (in 50% ethanol)

Initial concentration, $C_0 = 4.693 \times 10^{-4}$(M), Temperature 30°C

λ (nm)	Time (hour)	O.D.	C_t(M)	$\log \frac{C_0}{C_0 - C_t}$	K_{hyd} (min^{-1})	Average K_{hyd}(min^{-1})	$T_{\frac{1}{2}}$(hr.)
294	0	0.12	-	-			
	240	0.249	0.339×10^{-4}	0.052	0.83×10^{-5}		
298	0	0.08	-	-		0.79×10^{-5}	147
	240	0.222	0.5×10^{-4}	0.1489	0.78×10^{-5}		
300	0	0.07	-	-	0.78×10^{-5}		
	240	0.22	0.302×10^{-4}	0.149			

Experiment - 26

Antifungal activity of SR-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} =	15.00	19.82	23.43	24.16
ED_{50} =	1.32	2.35	3.14	4.24
Regression constants : Y = mX + C				
m =	45.34	49.81	51.59	59.54
c =	41.65	31.31	24.33	12.65
r =	0.998	0.992	0.996	0.999

*Data used for regression analysis.

Experiment - 27

Antifungal activity of [illegible] 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	94.50*	90.00*
10	96.25*	82.50*	76.75	72.60*
5	85.40*	71.00*	66.00*	62.40*
2.5	73.00*	59.20*	53.40*	48.00*
1.25	61.00*	46.00*	37.50*	29.00*
0.625	43.60*	34.75*	25.40*	18.00*
ED_{95} =	8.63	20.18	24.50	27.06
ED_{50} =	0.67	1.31	2.27	3.03
Regression constants : Y = mX + C				
m =	43.66	43.03	43.53	47.37
c =	55.44	42.76	34.46	27.15
r =	0.999	0.999	0.998	0.998

* Data used for regression analysis.

Experiment - 28

Antifungal activity of BR-9 against *M. gypseum* (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	97.00*	90.00*	85.00*
10	85.50*	74.00*	67.00*	62.20*
5	75.00*	66.75*	60.40*	55.00*
2.5	64.75*	54.00*	44.00*	40.60*
1.25	50.50*	40.00*	35.20*	28.00*
0.625	33.00*	21.00*	15.20*	10.00*
ED_{95} =	14.82	18.40	38.17	40.35
ED_{50} =	1.33	2.39	3.38	4.03
Regression constants : Y = mX + C				
m =	43.02	62.85	42.76	45.04
c =	44.63	24.01	27.33	22.67
r =	0.992	0.843	0.986	0.987

* Data used for regression analysis.

Experiment- 29

Antifungal activity of BR-10 against *B. arrhiza* (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} =	10.13	18.90	23.99	51.37
ED_{50} =	1.30	1.66	2.27	3.15
Regression constants : Y = mX + C				
m =	44.76	42.68	43.99	37.13
c =	49.80	40.51	34.20	37.48
r =	0.995	0.998	0.993	0.999

* Data used for regression analysis.

Experiment 30

Antifungal activity of BR-12 against *B. [illegible]* (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	100.00	100.00
10	100.00	100.00	100.00	90.25*
5	90.00*	90.50*	85.40*	80.75*
2.5	85.00*	80.00*	70.00*	60.00*
1.25	75.00*	60.00*	60.00*	45.00*
0.625	50.50*	45.00*	35.00*	25.00*
ED_{95} =	3.96	4.30	6.14	8.02
ED_{50} =	0.378	0.46	0.97	1.43
Regression constants : Y = mX + C				
m =	44.32	36.47	36.20	60.21
c =	68.26	39.39	50.70	40.54
r =	0.890	0.885	0.873	0.894

* Data used for regression analysis.

Experiment 31

Antifungal activity of BR-14 against *M. arrhiza* (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	98.00*	97.00*	85.00
10	100.00	85.50*	76.00*	66.00*
5	80.00*	72.50*	62.00*	58.50*
2.5	70.50*	64.20*	58.50*	52.50*
1.25	54.00*	41.00*	38.00*	26.00*
0.625	44.50*	36.75	22.20*	12.25
ED_{95} =	11.16	17.15	22.55	27.20
ED_{50} =	0.95	1.43	1.95	2.36
Regression constants : $Y = mX + C$				
m =	42.18	41.67	42.37	42.43
c =	50.81	43.37	34.67	34.13
r =	0.971	0.936	0.994	0.997

* Data used for regression analysis.

Experiment 32

Antifungal activity of BR-15 against *µ. [illegible]* (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	90.00*	88.30*	78.40*
10	100.00	80.80*	73.00*	60.40*
5	91.66*	64.40*	60.50*	50.03*
2.5	87.50*	58.00*	48.50*	42.00*
1.25	75.00*	45.80*	35.00*	28.00*
0.625	35.75*	33.00*	14.00*	8.80
ED_{95} =	3.30	26.04	33.30	60.46
ED_{50} =	0.46	1.69	2.80	5.00
Regression constants : Y = mX + C				
m =	32.74	37.93	41.85	40.26
c =	67.64	41.30	31.29	23.22
r =	0.992	0.993	0.999	0.993

* Data used for regression analysis.

Experiment 33

Antifungal activity of BP-37 against *p. 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	98.00*	92.20*	86.20*
10	100.00	85.50*	76.00*	66.00*
5	80.00*	72.50*	62.00*	58.20*
2.5	70.50*	64.20*	38.50*	32.50*
1.25	54.00*	48.00*	38.00*	26.00*
0.625	44.50*	36.70*	22.20*	12.20*
ED_{95} =	11.16	17.15	22.55	27.20
ED_{50} =	0.95	1.43	1.95	2.36
Regression constants : Y = mX + C				
m =	42.18	41.67	42.37	42.43
c =	50.91	43.57	37.67	34.13
r =	0.998	0.998	0.999	0.999

* Data used for regression analysis.

Experiment- 34

Effect of Hanosan on growth of *B. arvense* .

Concentration (µg/ml)	Percentage of inhibition over control after			
	24 hrs	48 hrs	72 hrs	96 hrs
500	100	100	100	100
400	100	100	100	100
300	100	100	97.50*	91.17
250	100	100	90.47*	86.76*
200	100	91.83*	83.33*	77.94*
150	80.30*	79.59*	73.80*	67.64*
100	75.00*	65.30*	59.52*	52.30*
50	56.00*	40.50*	29.30*	24.30*
ED_{95} =	180.20	218.77	269.15	309.92
ED_{50} =	39.81	63.09	81.28	93.32
Regression constants : Y = mX + C				
m =	60.92	83.58	86.13	86.56
c =	-87.56	-101.21	-114.54	-121.98
r =	0.997	0.999	0.998	0.999

* Data used for regression analysis.

Experiment- 35

Antifungal activity of BR-4 against *A. niger* (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*	84.60*	82.00*
1.5	93.30*	84.00*	72.30*	62.40*
0.75	78.00*	63.40*	51.40*	43.20*
0.375	54.40*	48.00*	42.30*	36.00*
ED_{95} =	1.31	2.31	4.11	6.23
ED_{50} =	0.30	0.41	0.58	0.82
Regression constants : Y = mX + C				
m =	64.93	59.40	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.

Experiment - 36

Antifungal activity of BR-8 against *F. oxysporum* (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	96.50*
6	100.00	100.00	95.00*	82.75*
3	92.50*	85.20*	78.75*	67.75*
1.5	80.50*	76.75*	70.40*	60.25*
0.75	66.40*	55.95*	49.75*	35.50*
0.375	45.90*	35.50*	24.50*	15.50*
ED_{95} =	3.38	3.85	6.00	10.85
ED_{50} =	0.40	0.61	0.86	1.40
Regression constants : Y = mX + C				
m =	51.06	36.45	33.49	30.70
c =	70.07	61.91	53.37	42.50
r =	0.99	0.96	0.98	0.98

* Data used for regression analysis.

Experiment - 3J

Antifungal activity of BR-9 against *A. 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	96.40*	88.30*
6	100.00	93.50*	86.30*	78.00*
3	96.50*	82.00*	75.00*	64.30*
1.5	80.00*	71.40*	62.80*	48.80*
0.75	63.80*	52.40*	46.20*	32.40*
0.375	44.50*	36.00*	29.00*	18.40*
ED_{95} =	2.64	4.83	8.14	14.58
ED_{50} =	0.45	0.61	0.93	1.66
Regression constants : Y = mX + C				
m =	58.97	50.16	47.71	47.74
c =	70.14	60.47	51.49	39.51
r =	0.99	0.98	0.99	0.99

* Data used for regression analysis.

Experiment - 38

Antifungal activity of BR-10 against *F. oxysporum* (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	90.30*	80.40*
6	100.00	86.40*	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} =	3.72	7.70	9.85	21.34
ED_{50} =	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- 39

Antifungal activity of BR-12 against *F. oxysporum* (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	96.90*
3	100.00	100.00	91.80*	84.60*
1.5	96.30	90.70*	69.40*	63.60*
0.75	85.50*	72.00*	58.90*	47.50*
0.375	66.20*	42.90*	24.60*	16.50*
ED_{95} =	1.41	1.61	3.20	4.14
ED_{50} =	0.16	0.44	0.74	0.93
Regression constants : Y = mX + C				
m =	47.01	79.40	70.46	69.23
c =	87.94	78.46	59.38	52.28
r =	0.97	0.99	0.98	0.99

*Data used for regression analysis.

Experiment- 40

Antifungal activity of SB-14 against [illegible] (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	94.00*	84.00*
6	100.00	90.50*	83.60*	71.80*
3	92.60*	76.00*	69.30*	60.40*
1.5	76.30*	62.00*	38.60*	44.60*
0.75	59.00*	45.40*	40.00*	26.30*
0.375	38.00*	28.00*	16.00	8.00
ED_{95} =	4.46	6.95	10.30	17.31
ED_{50}	0.54	0.94	1.36	2.12
Regression constants : Y = mX + C				
m =	49.00	51.69	51.16	49.08
c =	63.17	51.46	43.19	33.96
r =	0.86	0.99	0.99	0.99

* Data used for regression analysis.

Experiment- 41

Antifungal activity of [illegible]-10 against *[illegible]* [illegible] (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	92.60	84.80
6	100.00	94.20	78.00	68.00
3	82.80	72.40	61.00	54.40
1.5	66.75	48.00	40.00	32.00
0.75	40.00	28.20	18.70	12.20
0.375	25.20	12.00	6.40	2.00
ED_{95} =	4.43	6.48	11.70	16.72
ED_{50} =	0.26	1.48	2.22	2.97
Regression constants : Y = mX + C				
m =	63.01	69.30	61.73	60.00
c =	54.26	38.76	28.42	21.60
r =	0.99	0.99	0.99	0.99

- Data used for regression analysis.

Experiment- 42

Antifungal activity of BR-17 against *A. niger* (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	98.60	86.00	76.60
6	95.80	80.50	74.50	62.60
3	80.40	64.90	58.90	40.90
1.5	68.40	48.00	45.00	31.00
0.75	41.00	28.90	24.90	10.90
0.375	27.00	8.00	3.00	-
ED_{95} =	0.76	9.86	14.51	23.49
ED_{50} =	0.84	1.73	2.21	3.49
Regression constants : Y = mX + C				
m =	53.48	39.60	35.02	34.15
c =	34.01	35.77	31.08	20.37
r =	0.99	0.99	0.99	0.99

* Data used for regression analysis.

FIGURE OF DIFFERENT SPECTRA

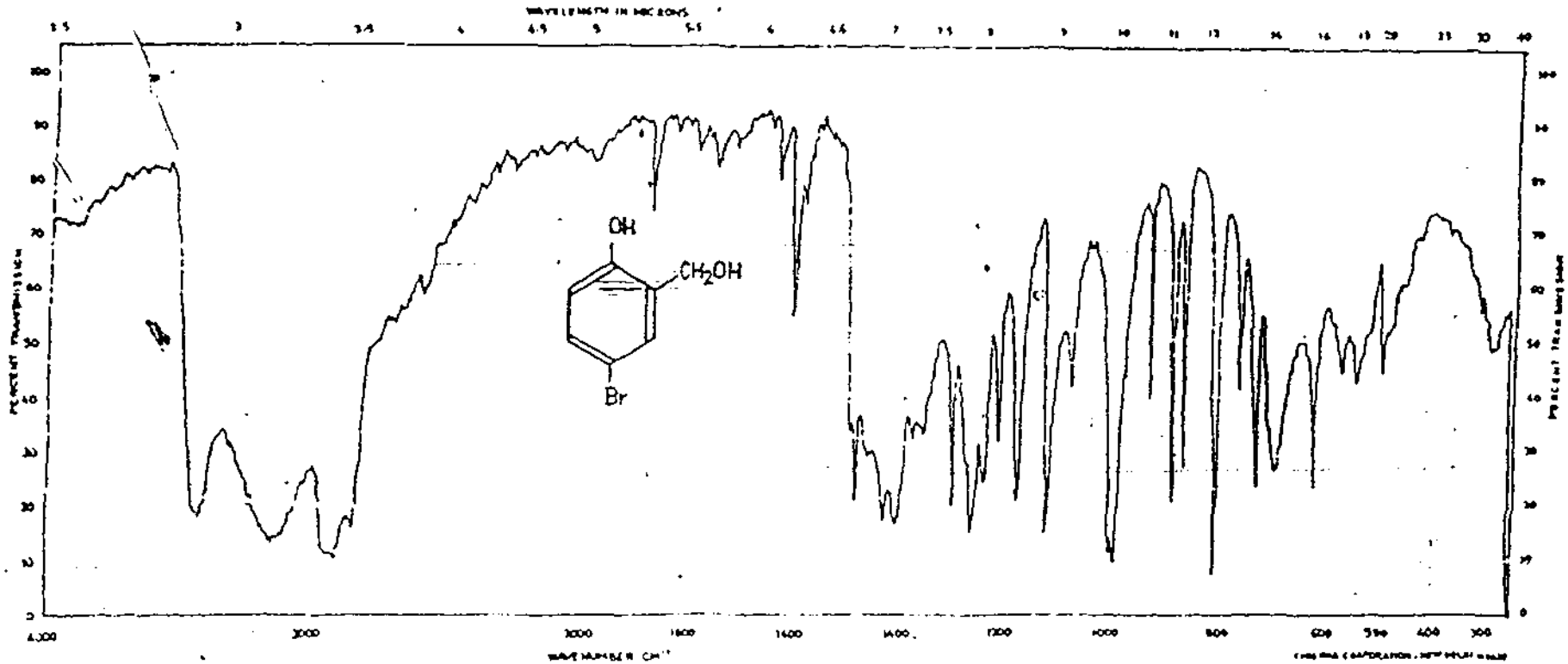


Fig.2.2: IR spectrum of 5-bromo-saligenin.

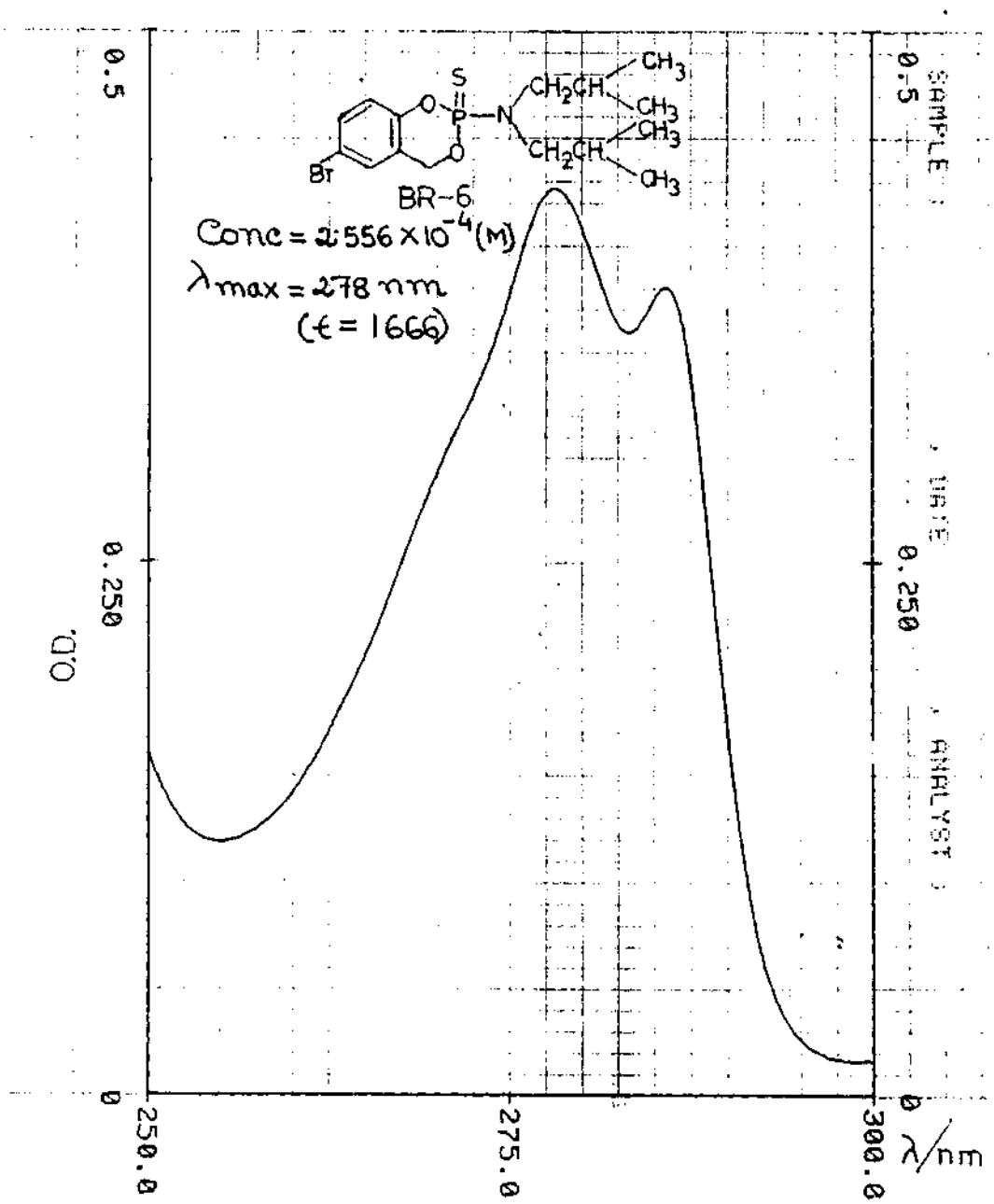


CHART 200-91522

Fig.2.3 : Electronic absorption spectrum of 2-N,N-Diisobutylamido-6-bromo-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BR-6).

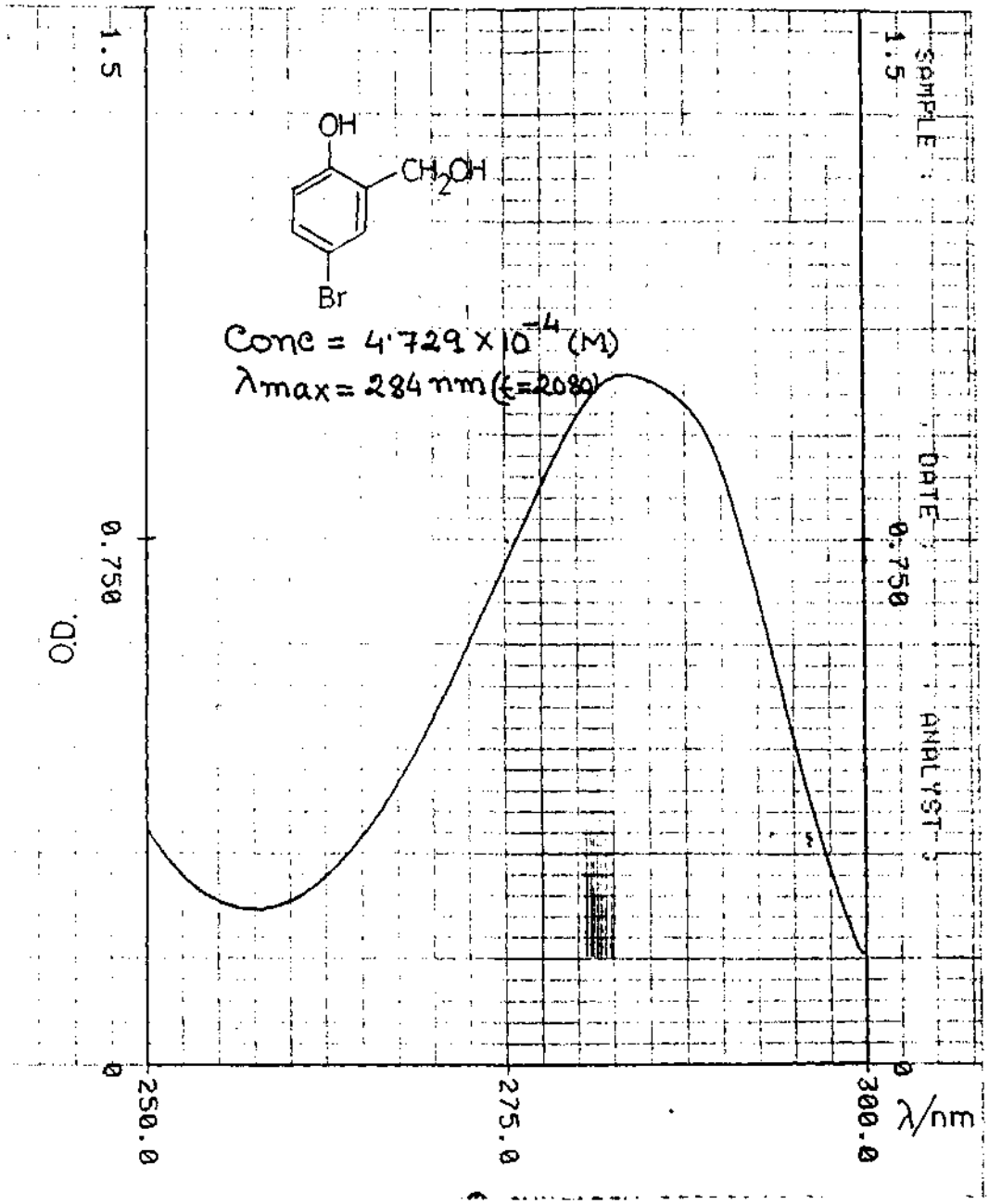


Fig. 2.1 : Electronic absorption spectrum of 5-bromo-saligenin.

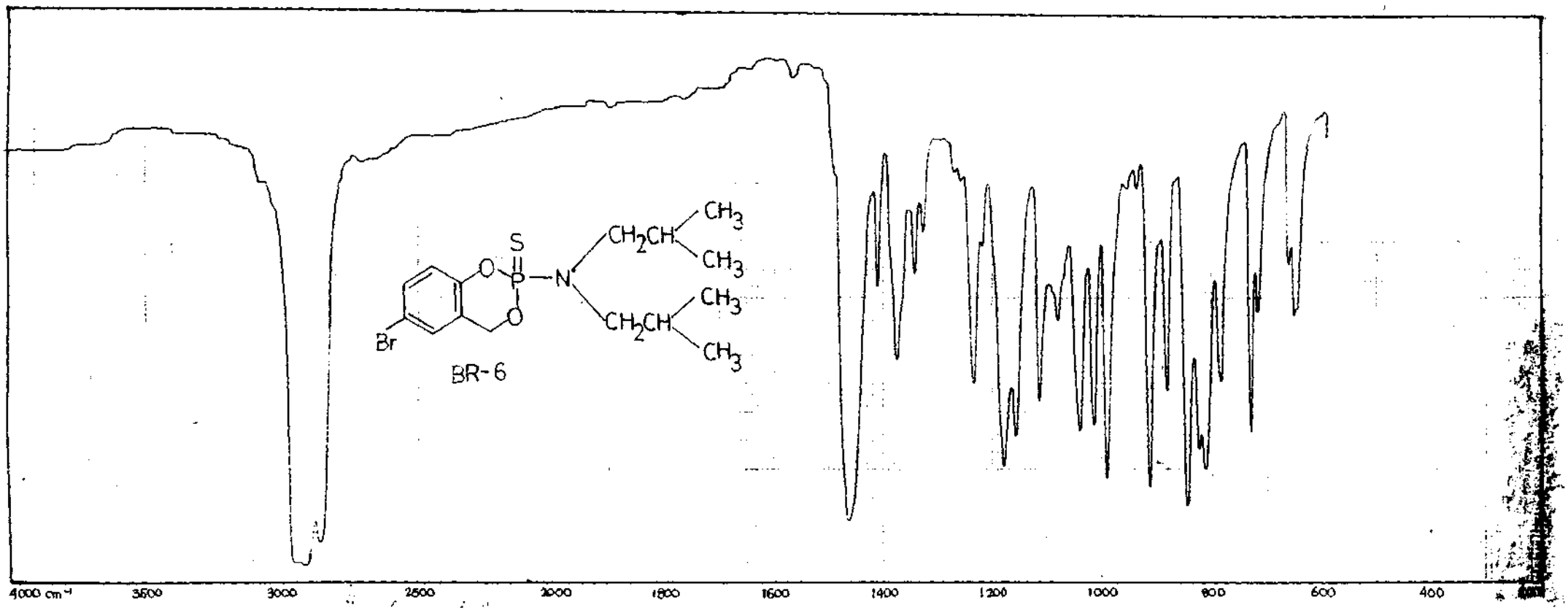


Fig.2.4 : IR spectrum of 2-N,N-Diisobutylamido-6-bromo-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BR-6).

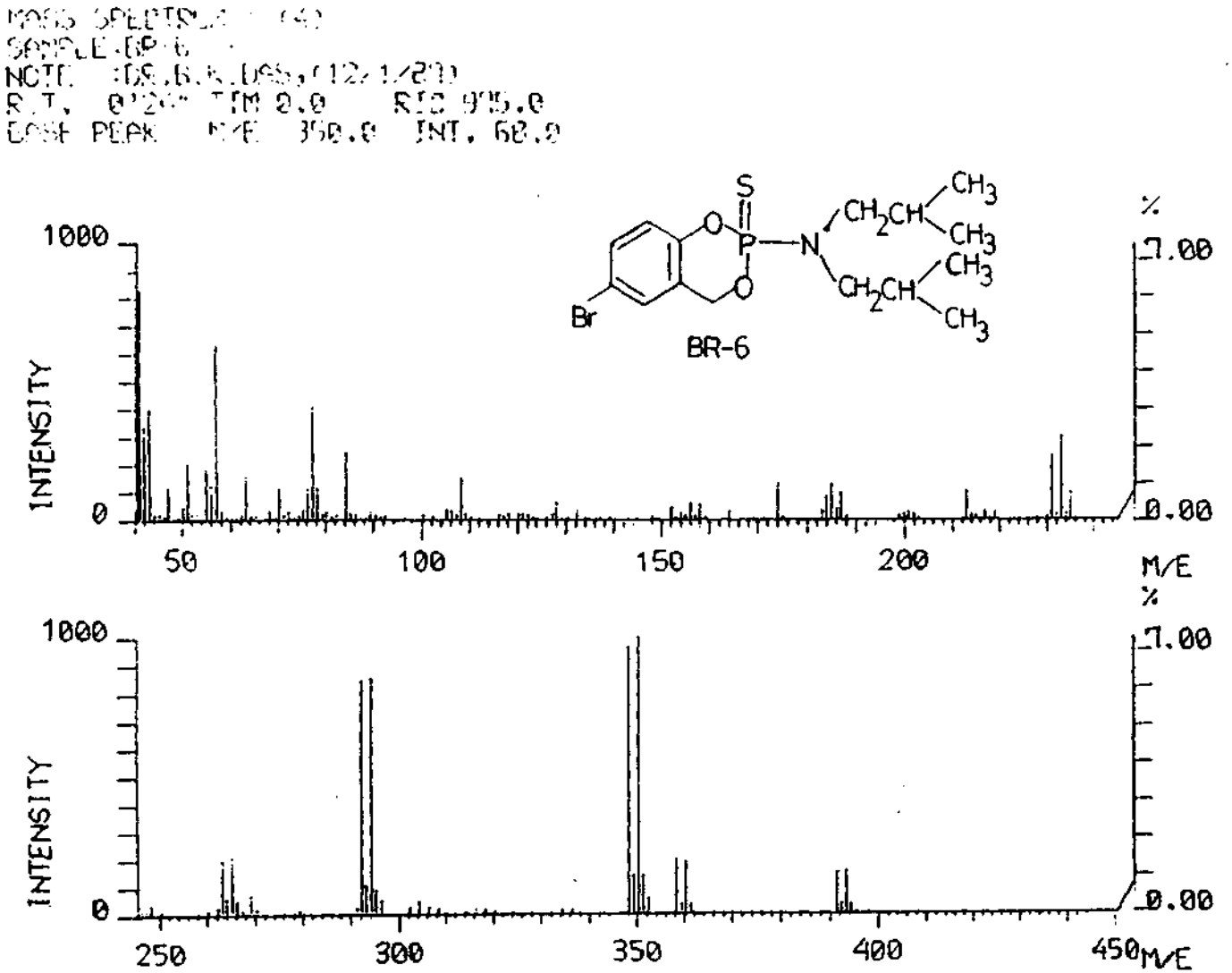


Fig.25: Mass spectrum of 2-N,N-Diisobutylamido-6-bromo-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BR-6).

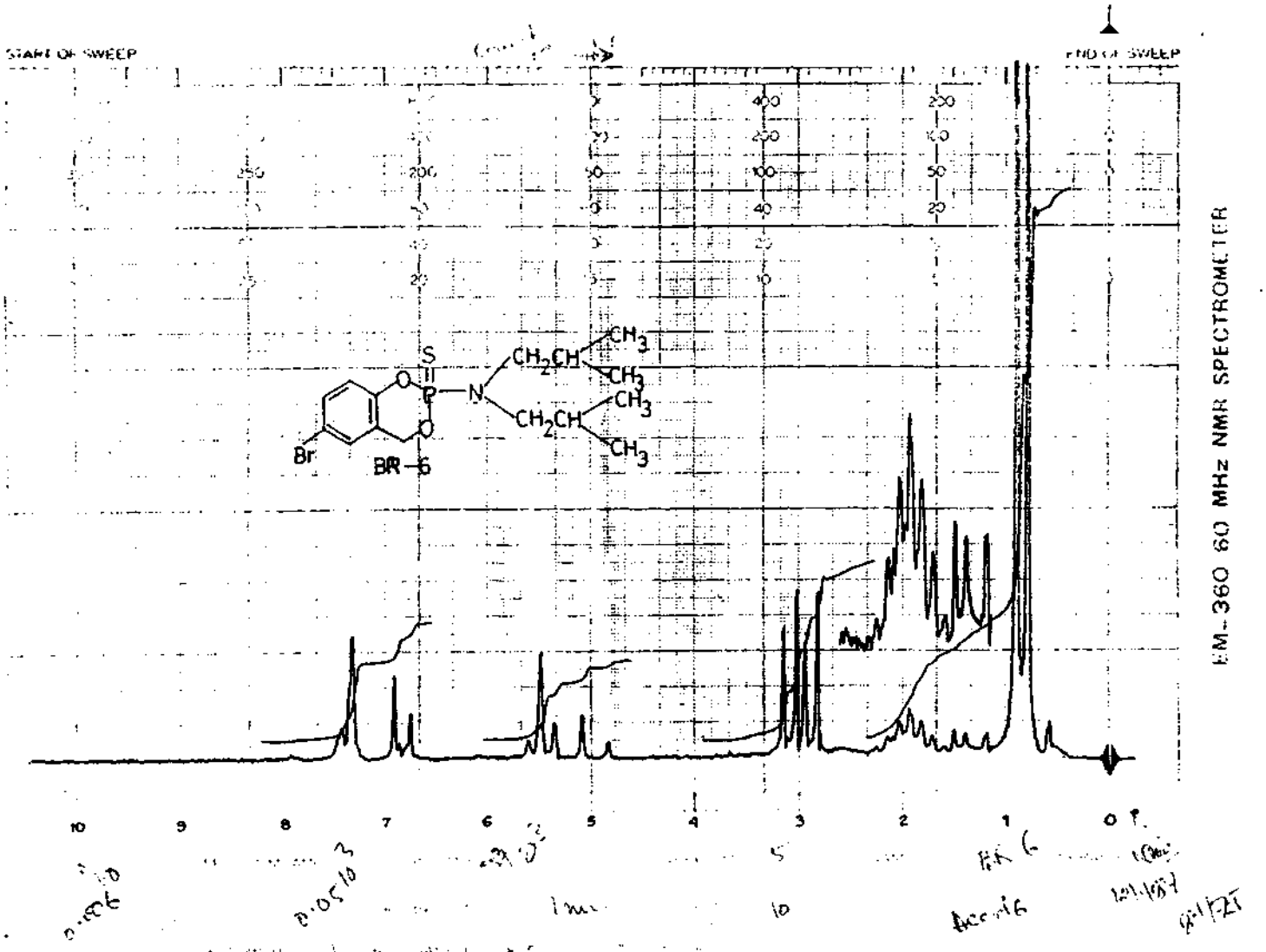
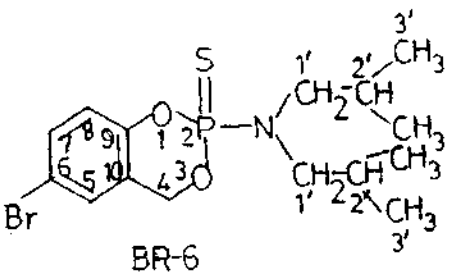


Fig.2.6: ^{1}H NMR spectrum of 2-N,N-Diisobutylamido-6-bromo-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BR-6).



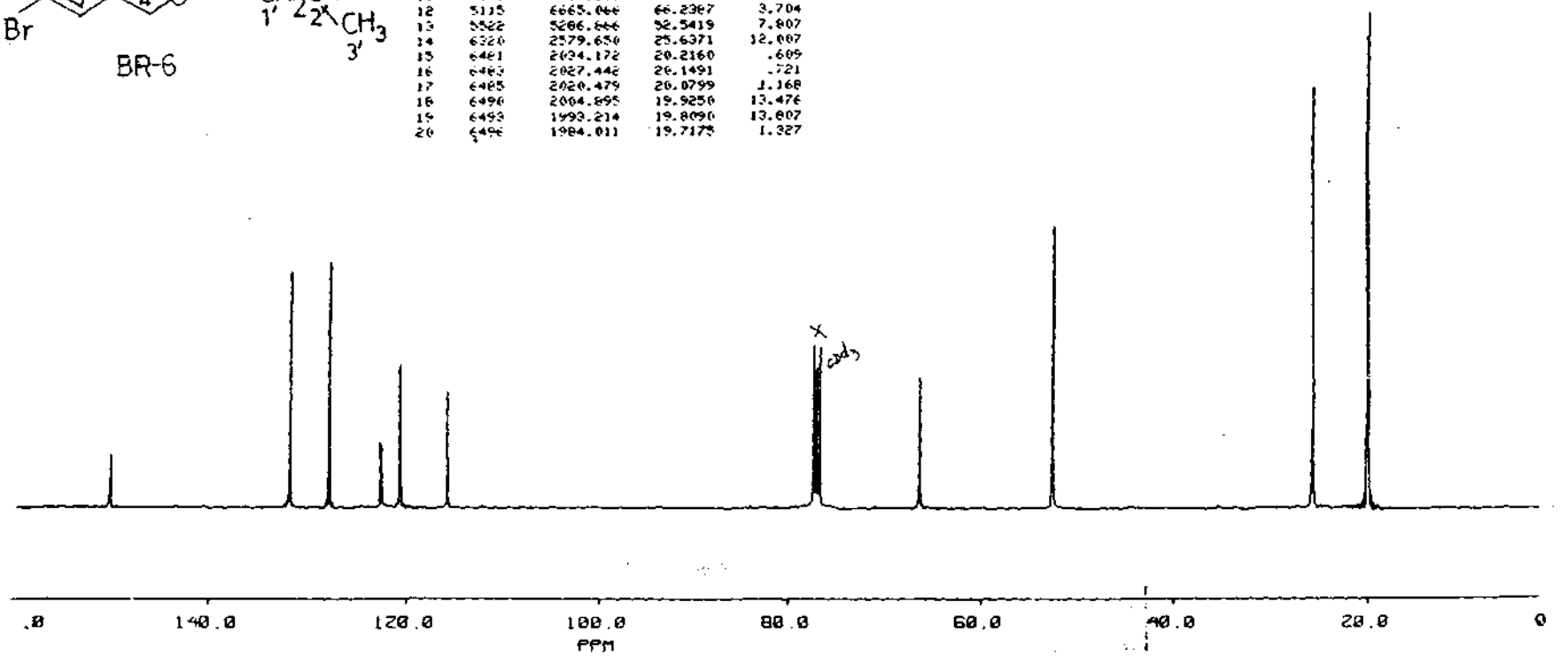


Fig. 2.7 . 100 MHz proton noise decoupled ^{13}C spectrum of 2-N,N-Diisobutylamido-6-bromo-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BR-6)

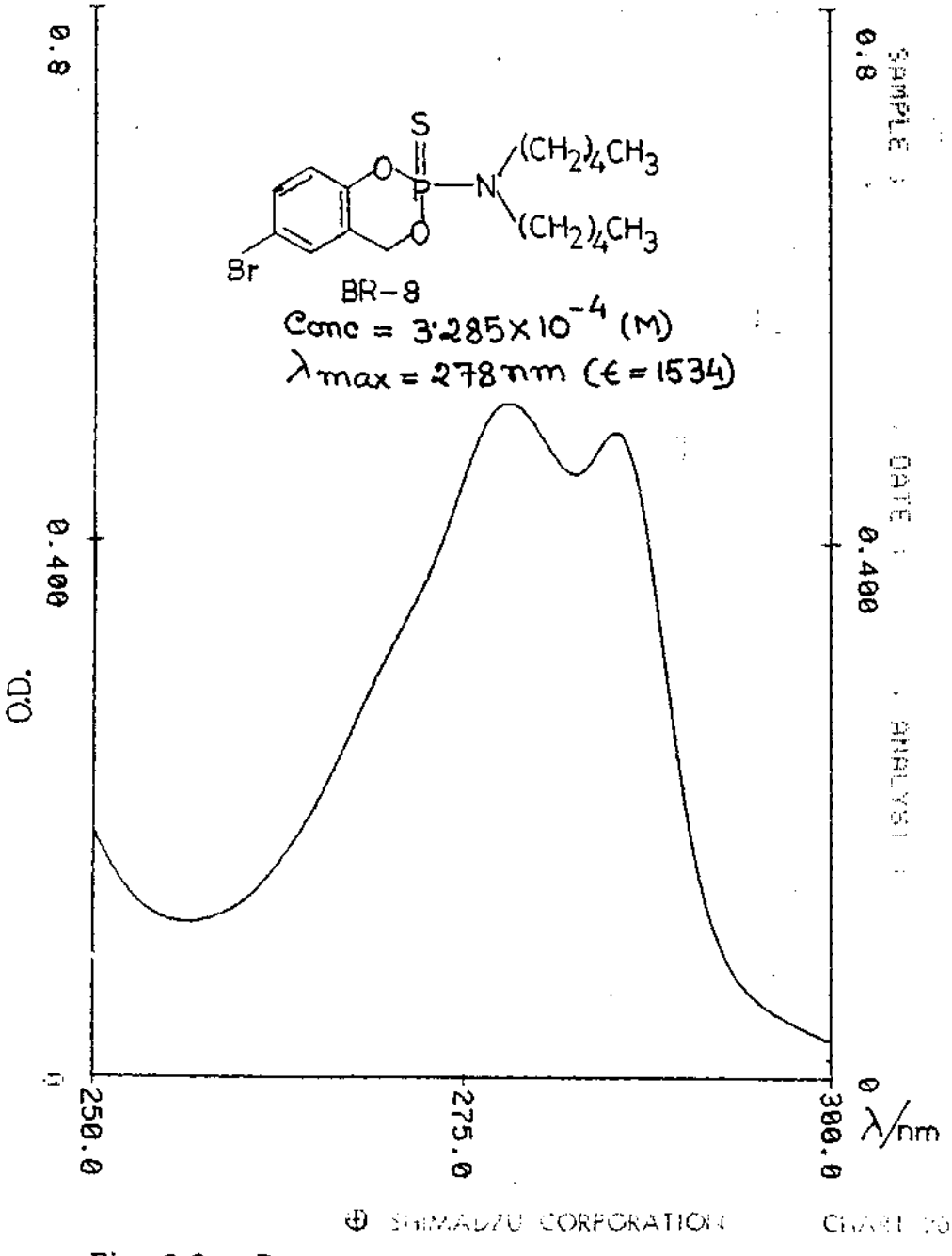


Fig.2.8 : Electronic absorption spectrum of 2-N,N-Dipentylamido-6-bromo-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BR-8).

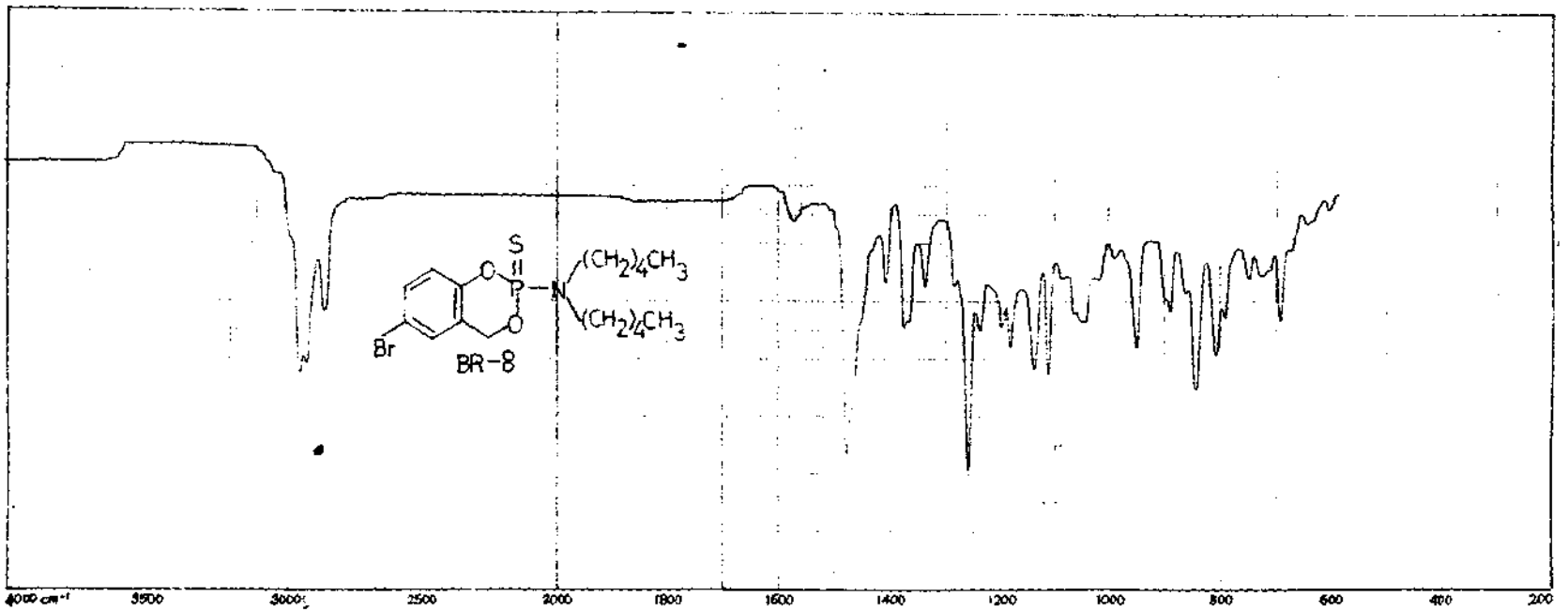


Fig.29: IR spectrum of 2-N,N-Dipentylamido-6-bromo-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BR-8).

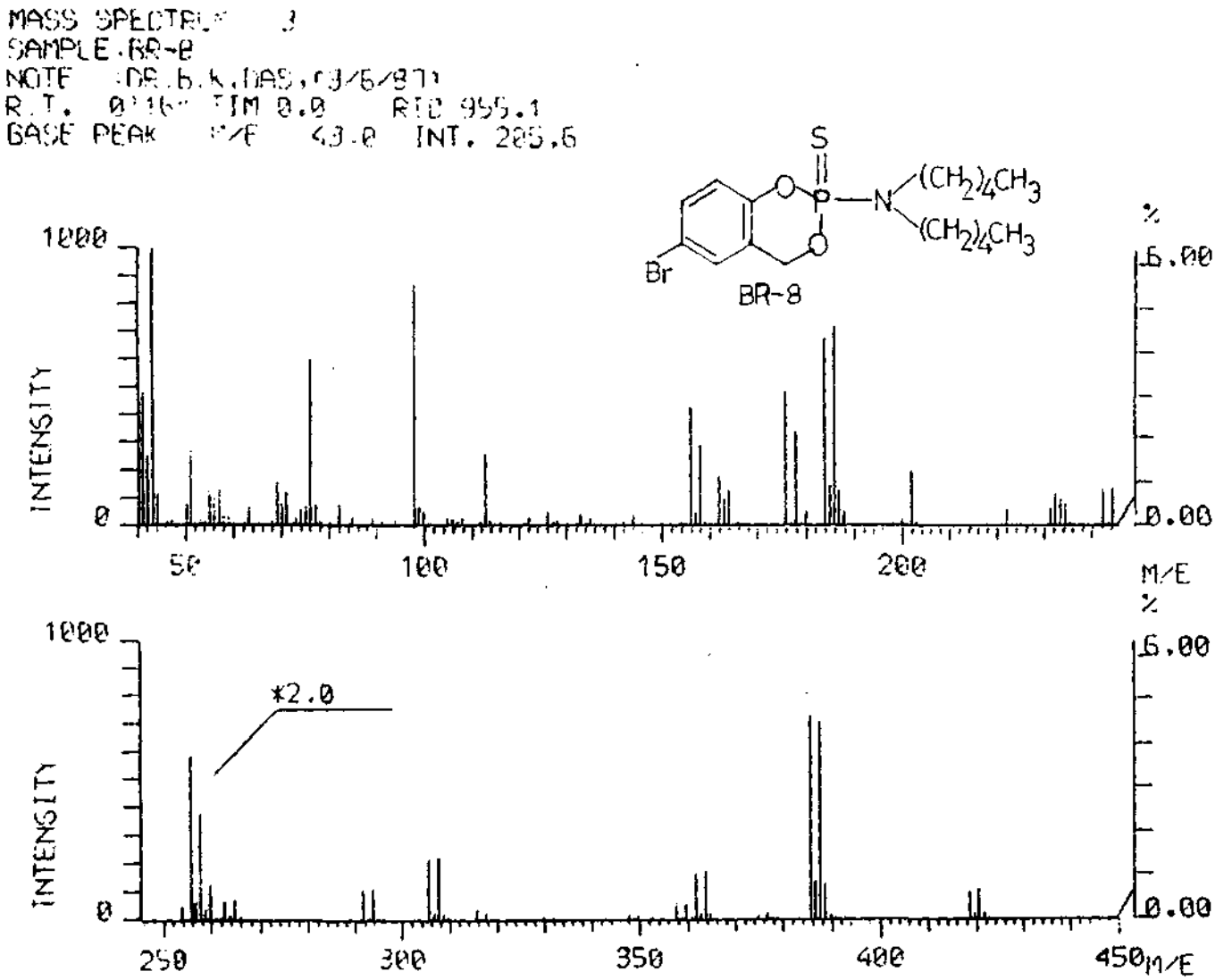


Fig.2.10: Mass spectrum of 2-N,N-Dipentylamido-o-bromo-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BR-8).

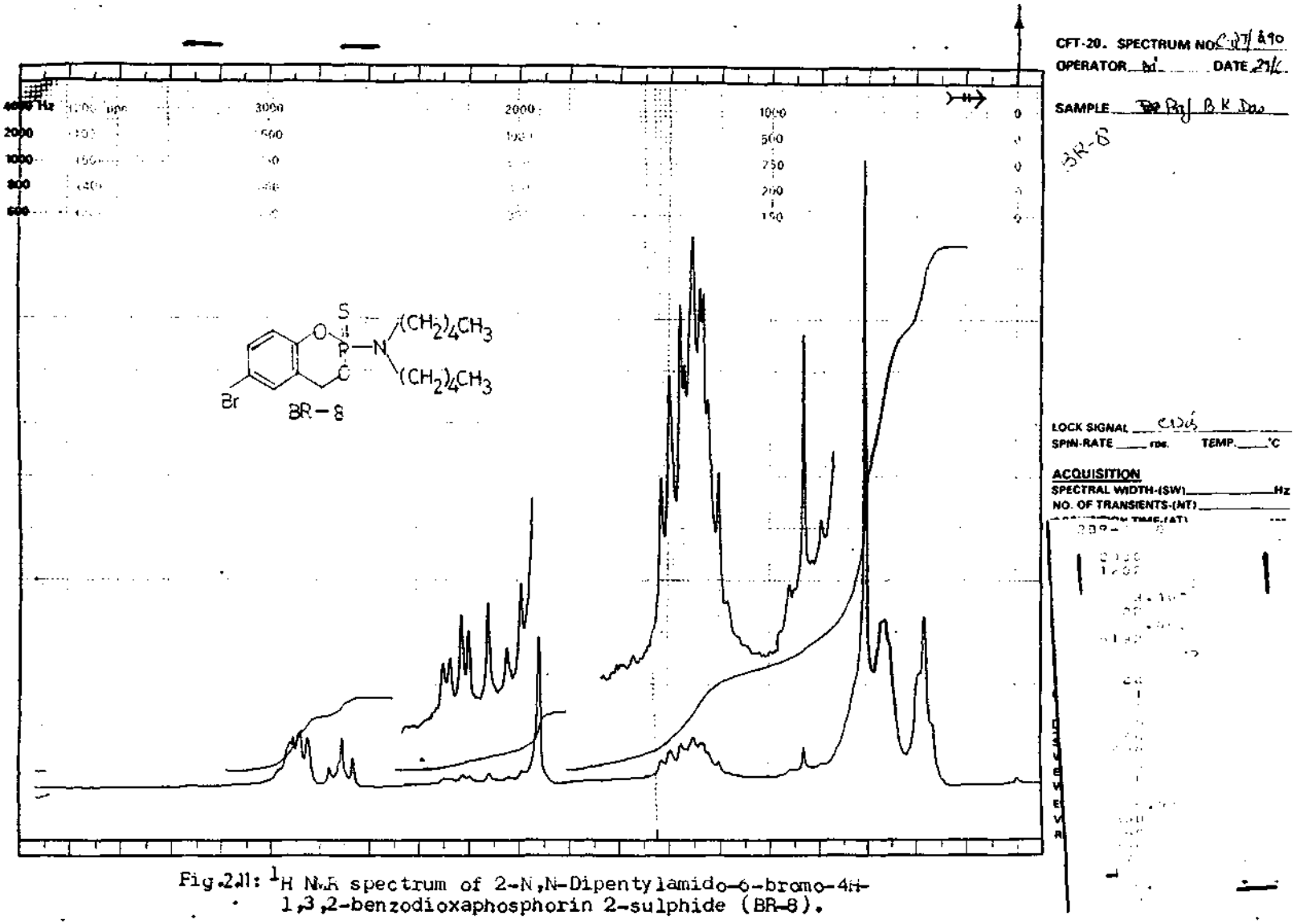


Fig.2.11: ^{1}H N.M.R spectrum of 2-N,N-Dipentylamido-6-bromo-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BR-8).

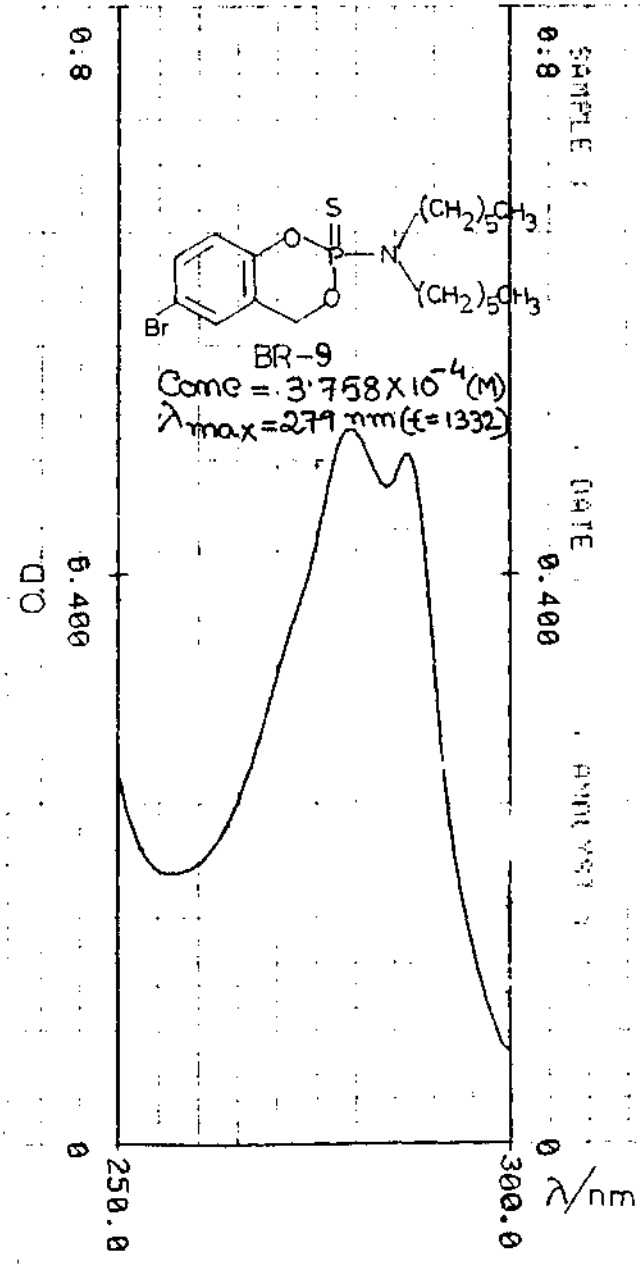


Fig.2.12 : Electronic absorption spectrum of 2-N,N-Dinexylamido-o-bromo-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BR-9).

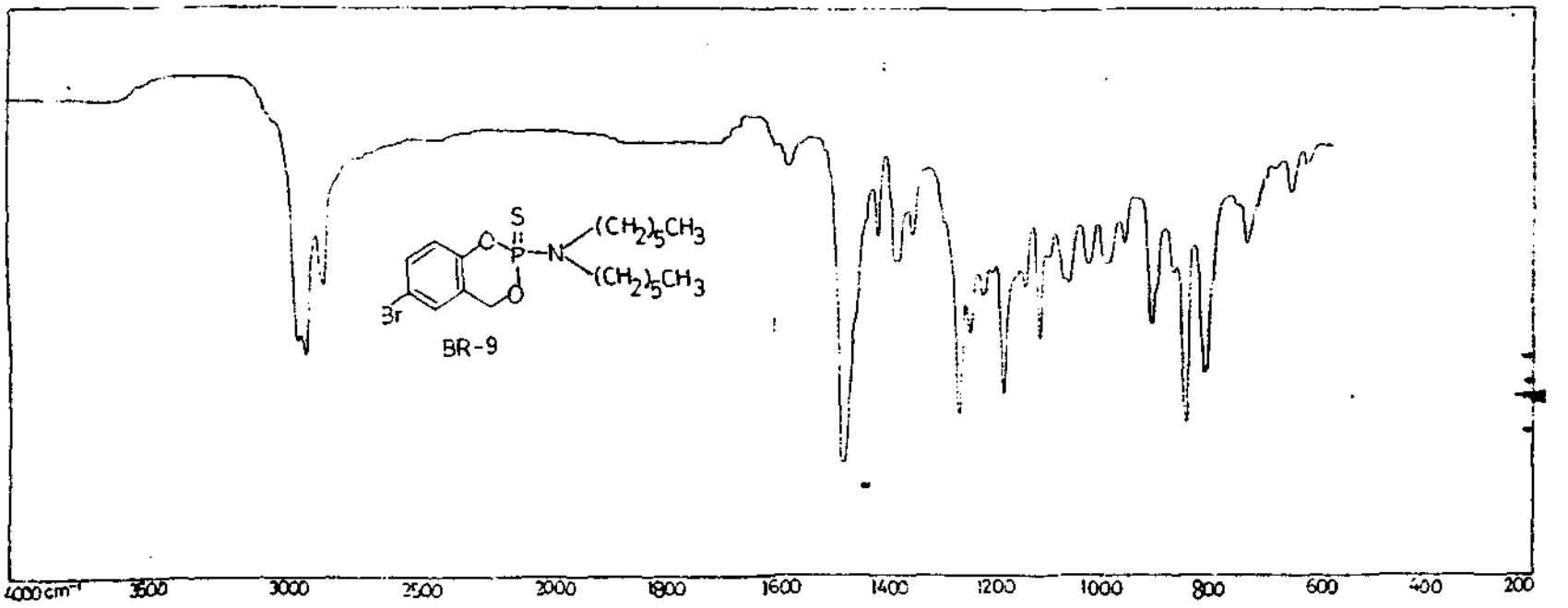


Fig.213: IR spectrum of 2-N,N-Dihexylamido-6-bromo-4H-1,3,2-benzo-dioxaphosphorin 2-sulphide (BR-9).

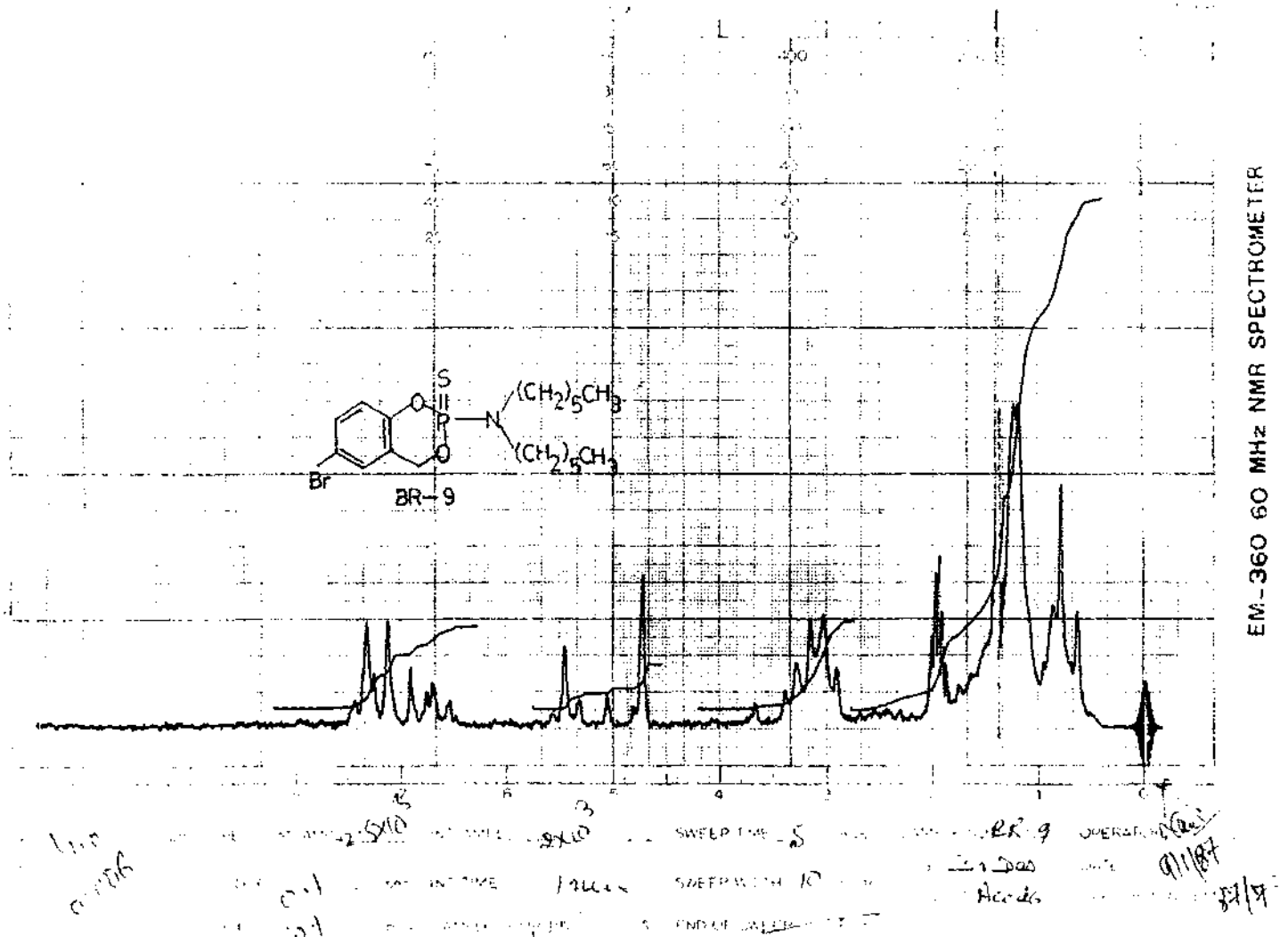


Fig 2.4: ^1H NMR spectrum of 2-N,N-Dihexylamido-6-bromo-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BR-9).

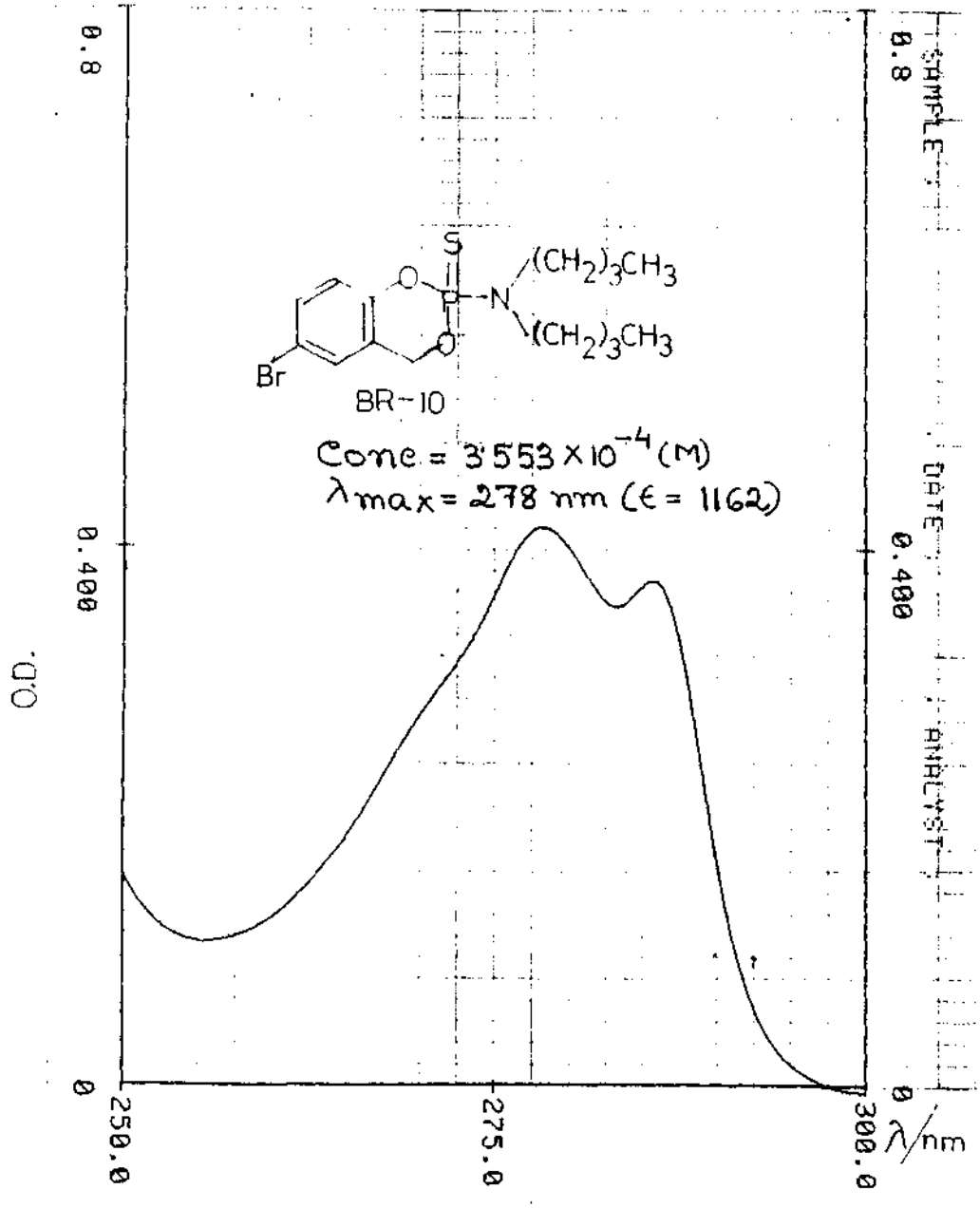


Fig.2.15 : Electronic absorption spectrum of 2-N,N-Dibutylamido-6-bromo-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BR-10).

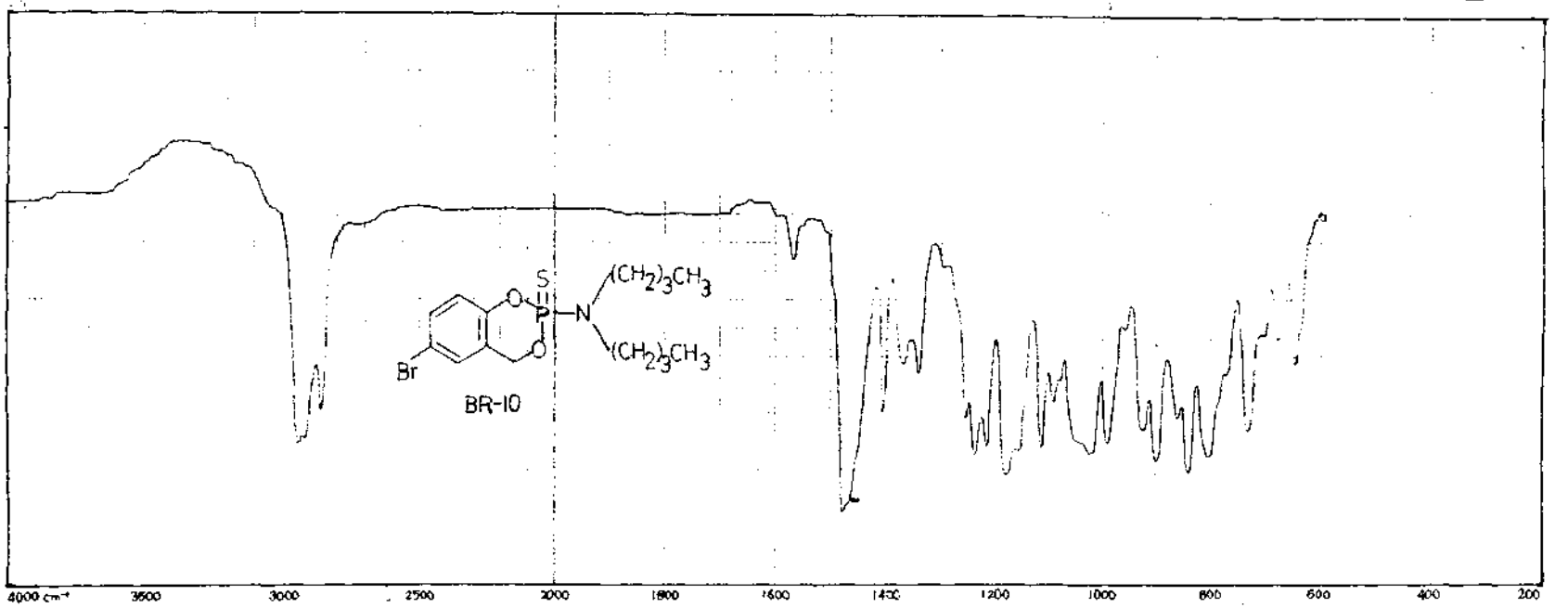


Fig.2.16 IR spectrum of 2-N,N-Dibutylamido-6-bromo-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BR-10).

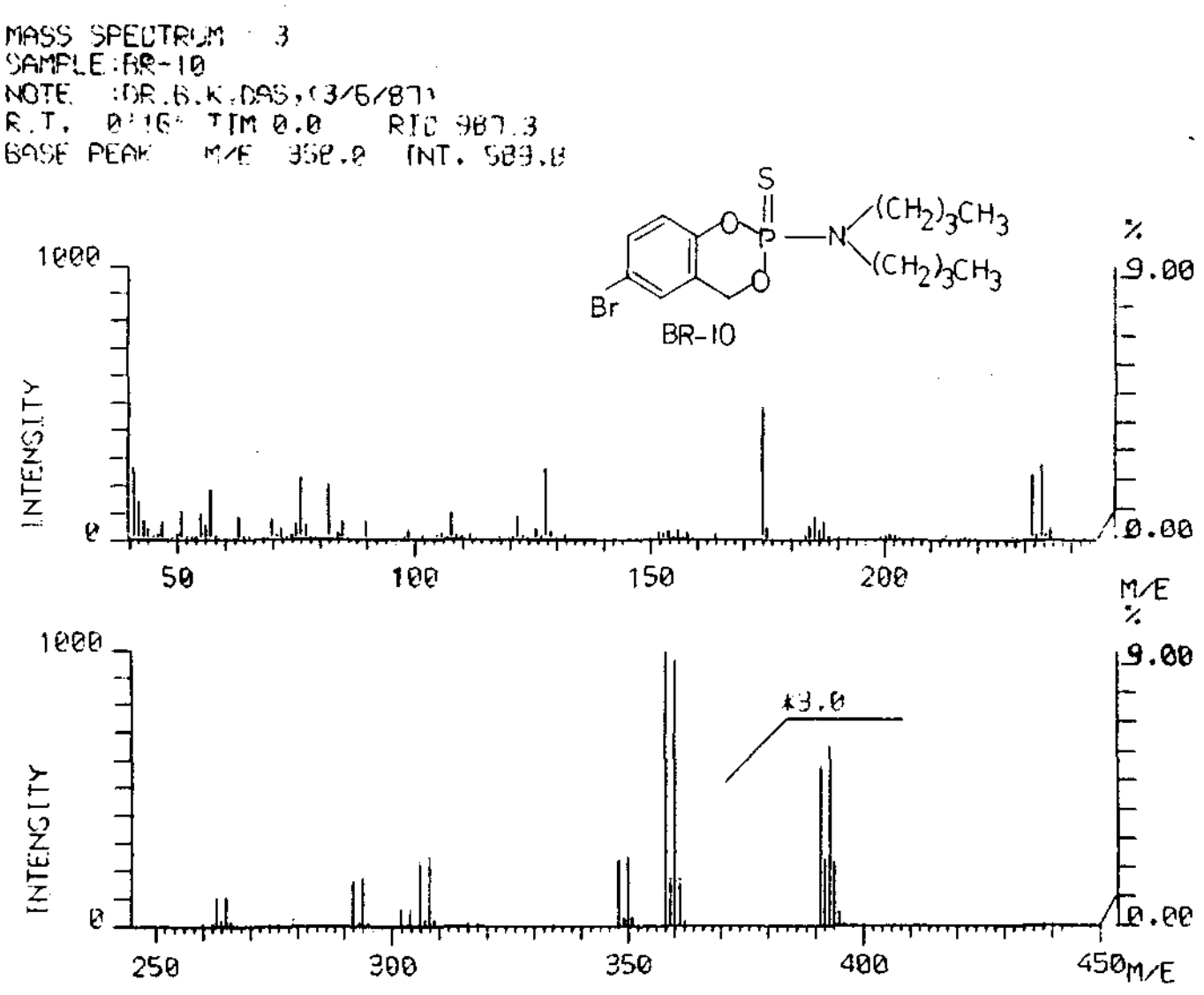


Fig 2.17: Mass spectrum of 2-N,N-Dibutylamido-o-bromo-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BR-10).

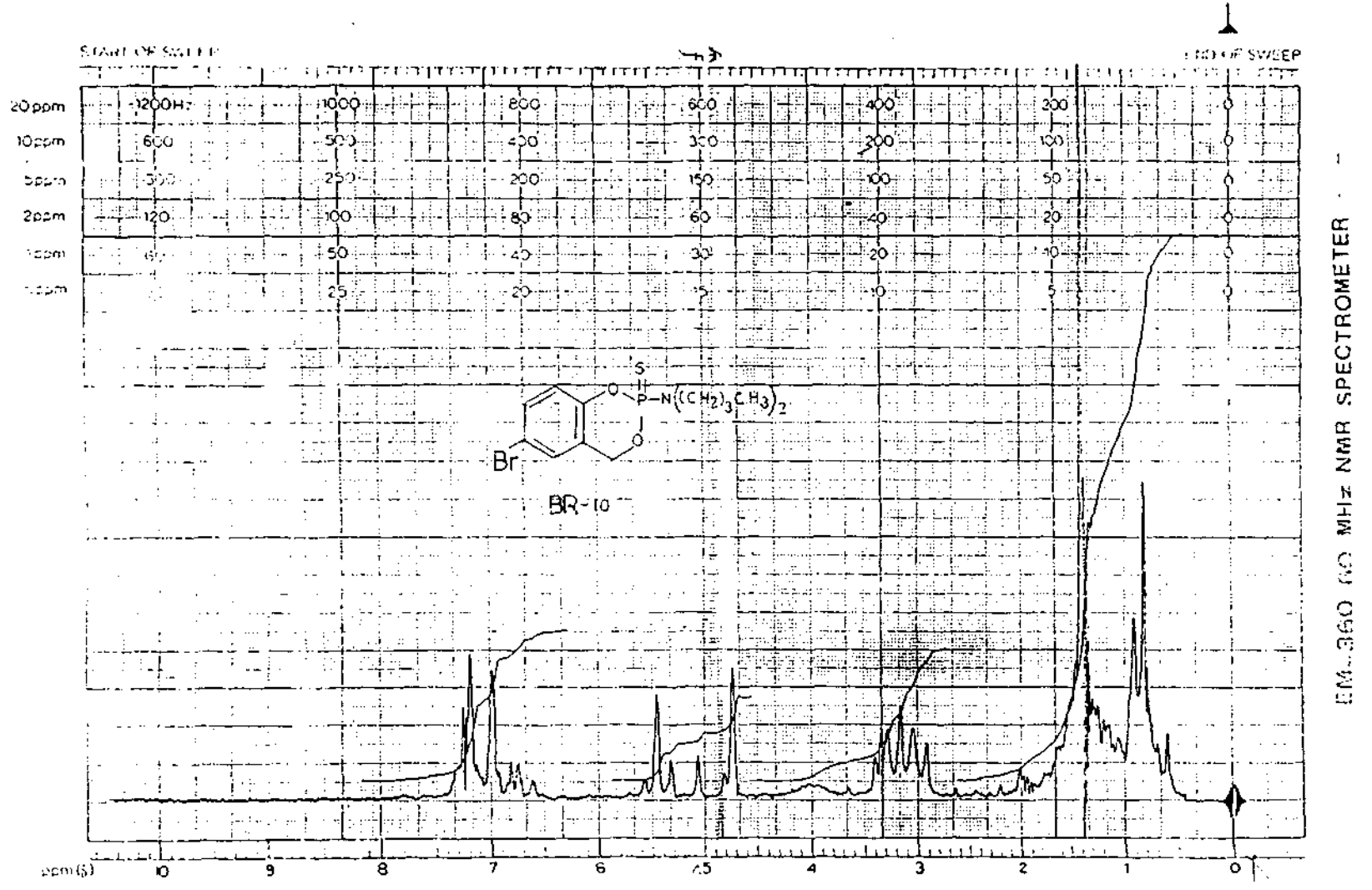


Fig 2.18: ^{1}H NMR spectrum of 2-N,N-Dibutylamido-6-bromo-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BR-10).

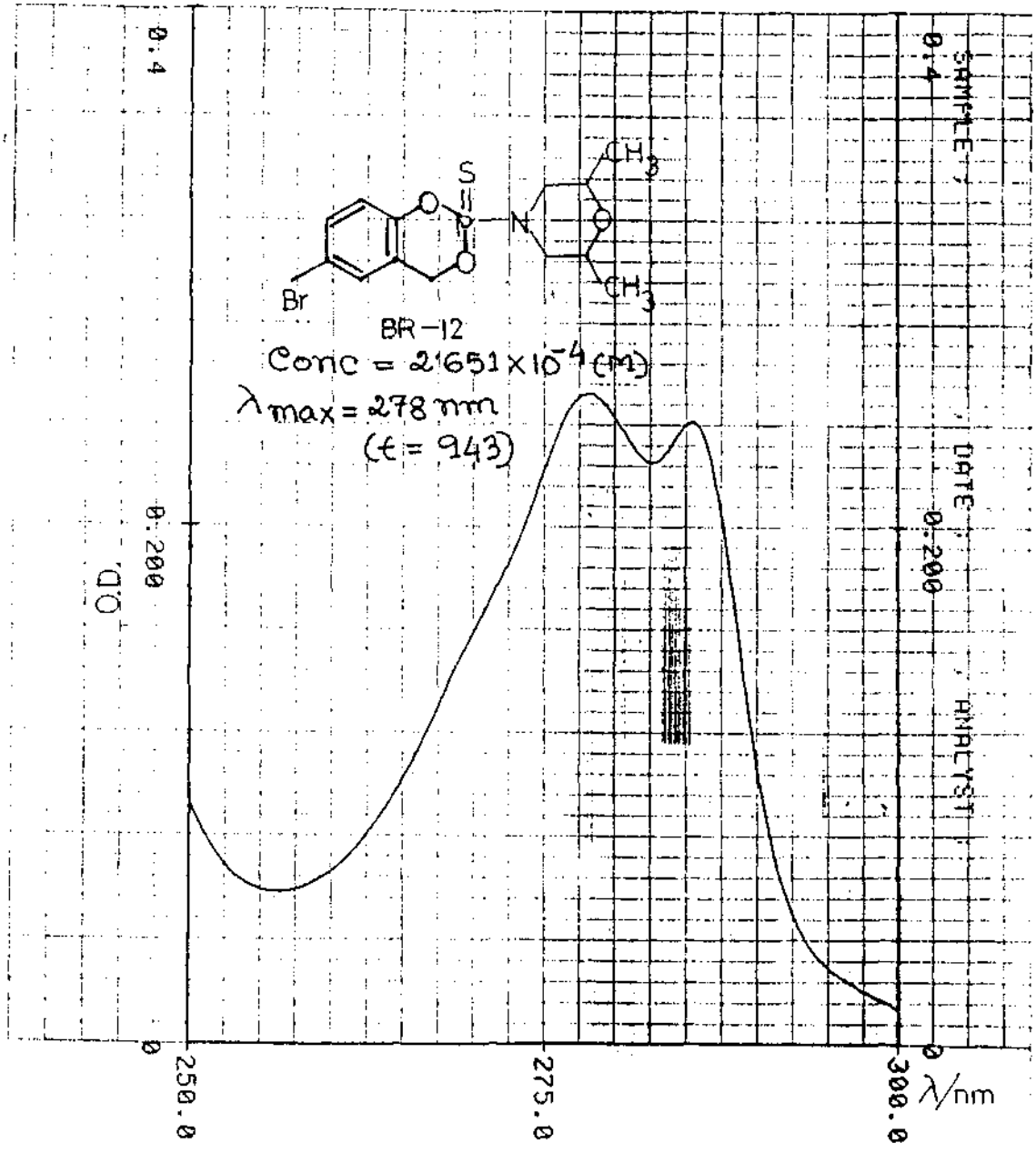


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Fig.2.19 : Electronic absorption spectrum of 2-(2,6-Dimethylmorpholino)-6-bromo-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BR-12).

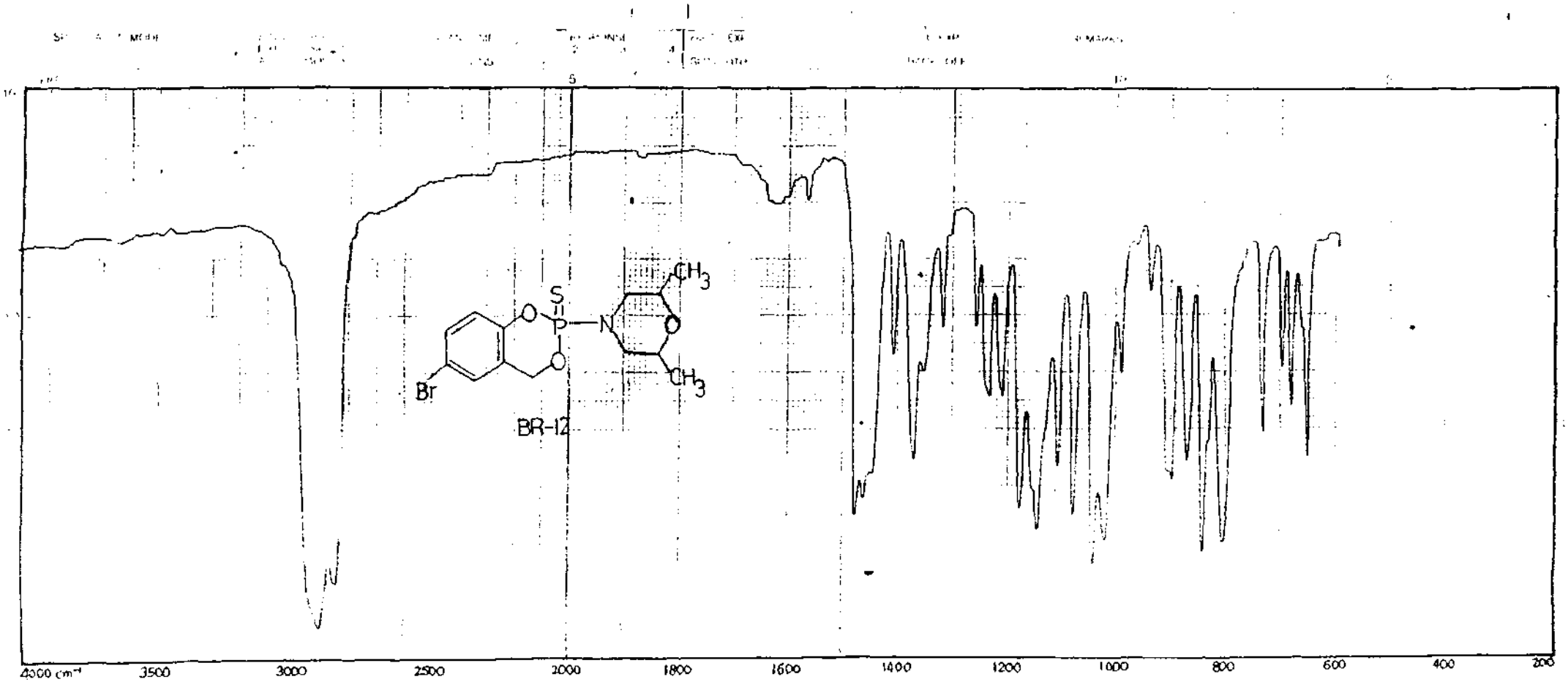


Fig.2.20:IR spectrum of 2-(2,6-Dimethylmorpholino)-6-bromo-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BR-12).

MASS SPECTRUM : 3
SAMPLE:BR-12
NOTE :DR.B.K.DAS,<2/5/88>
R.T. 0'16" TIM 0.0 RIC 905.4
BASE PEAK : M/E 364.0 INT. 391.3

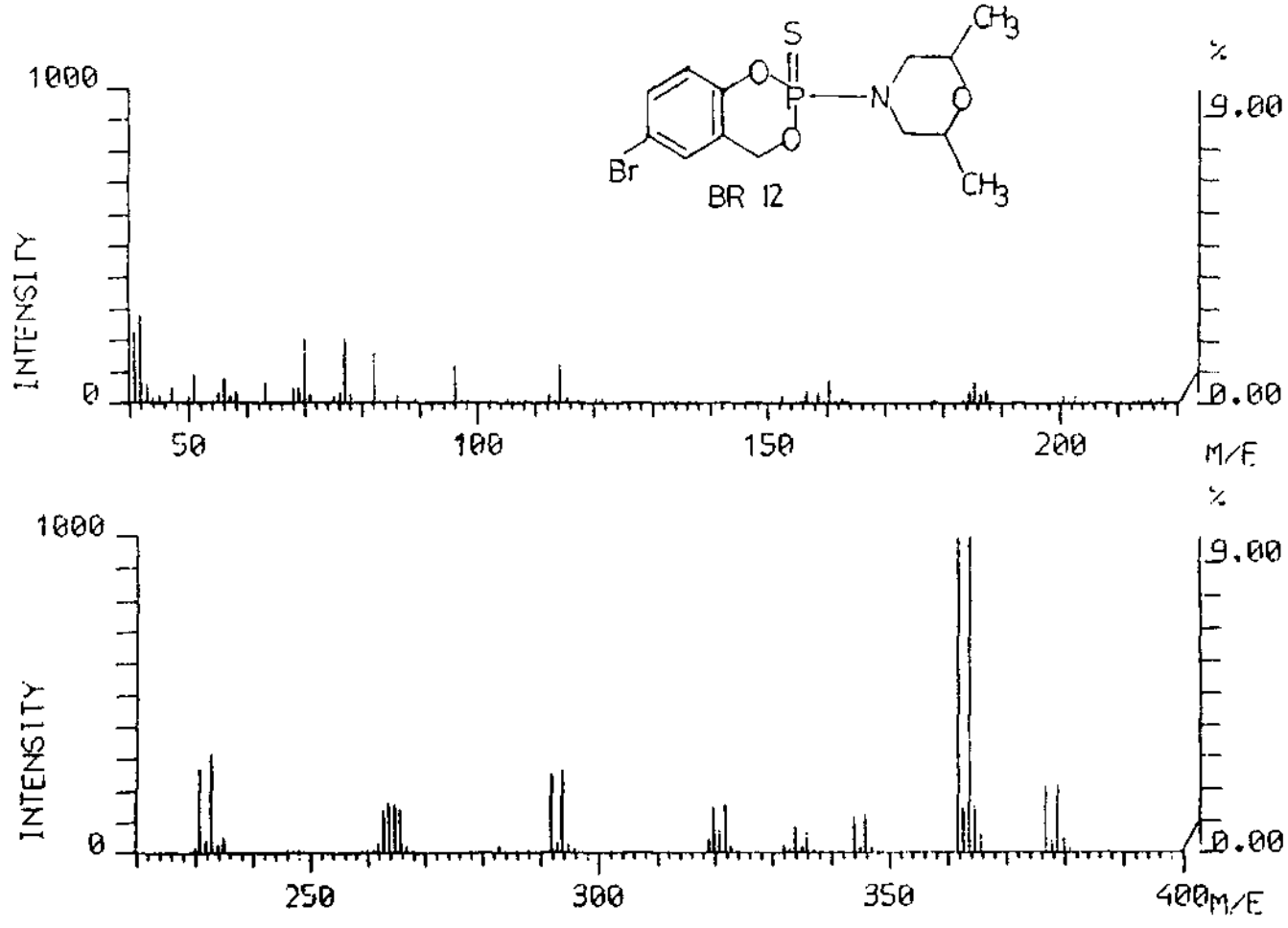


Fig 2.21: Mass spectrum of 2-(2,6-Dimethylmorpholino)-6-bromo-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BR-12).

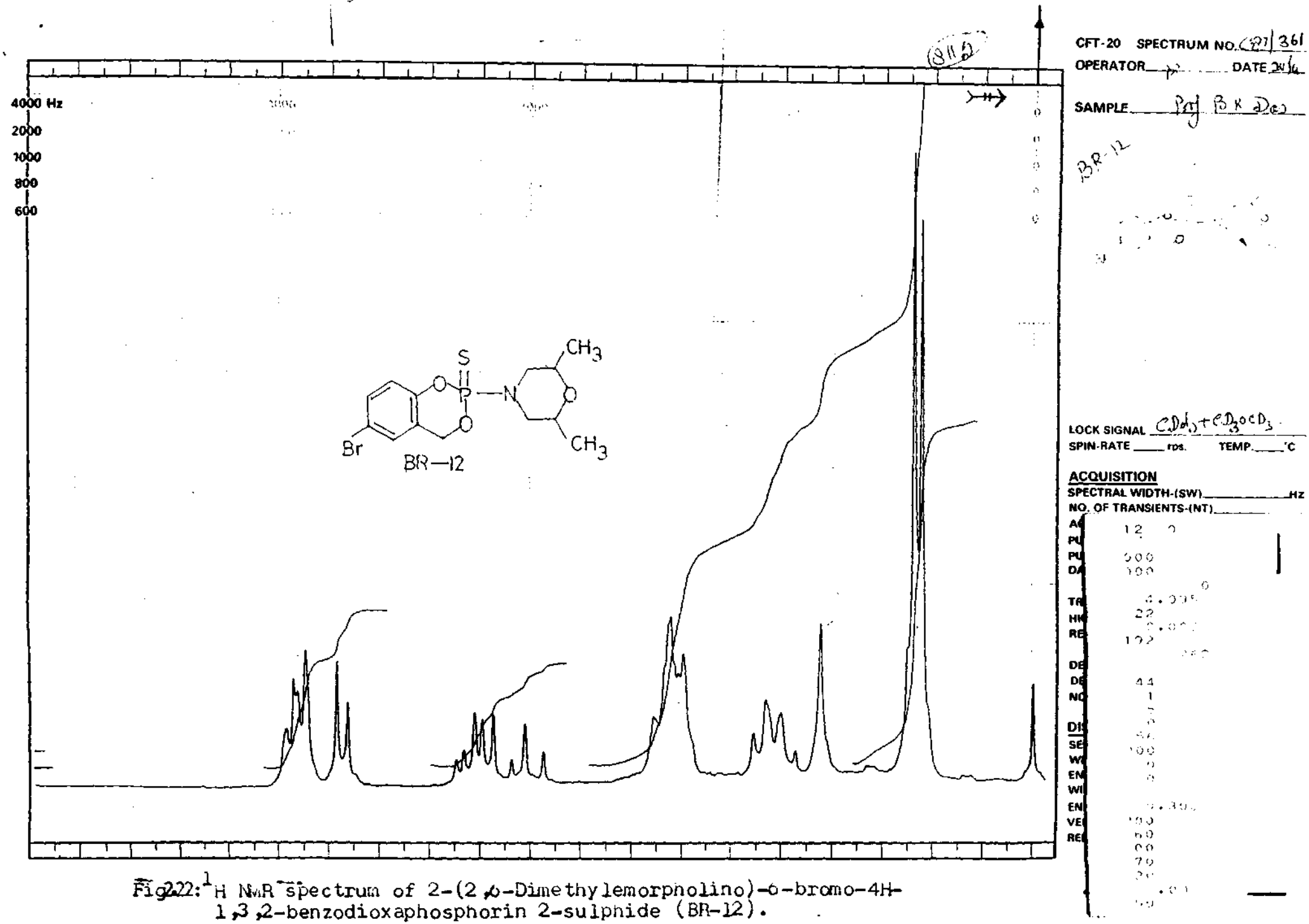


Fig.22: ^{1}H NMR spectrum of 2-(2,6-Dimethylemorpholino)-6-bromo-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BR-12).

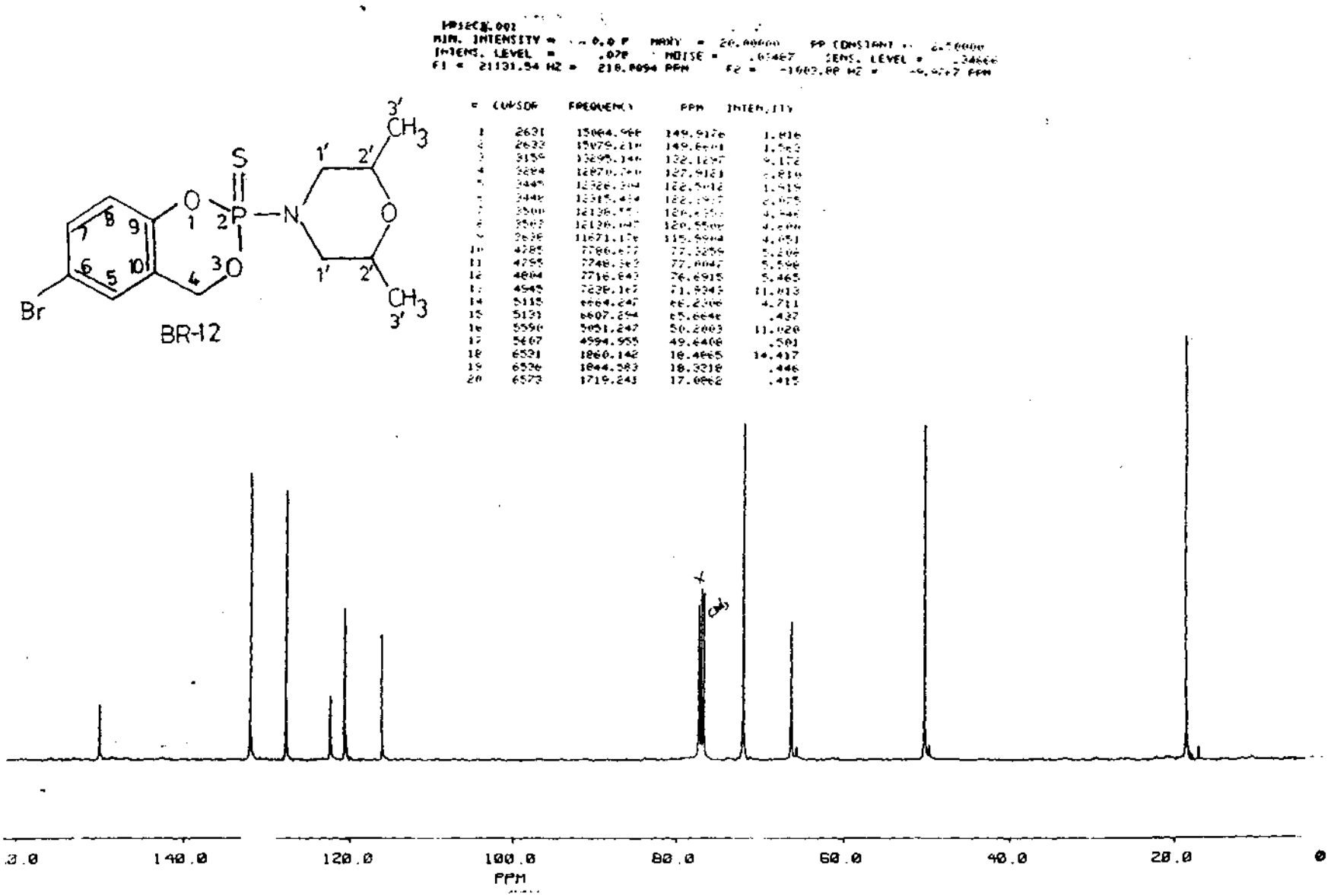


Fig.2.23. 100 MHz proton noise decoupled ^{13}C spectrum of 2-(2,6-Dimethylmorpholino)-6-bromo-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BR-12).

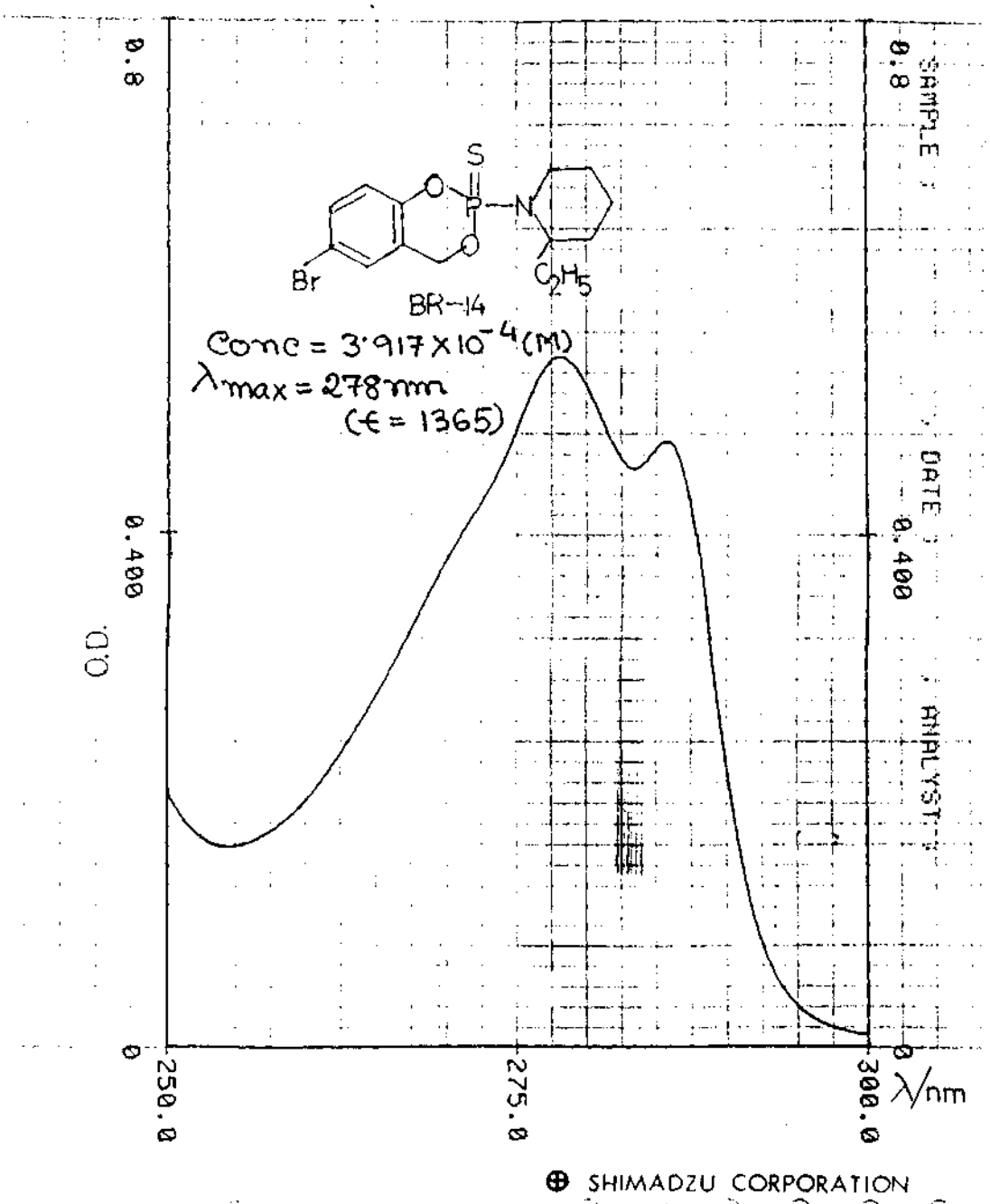


Fig.2.24: Electronic absorption spectrum of 2-(2-Ethyl-piperidino)-6-bromo-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BR-14).

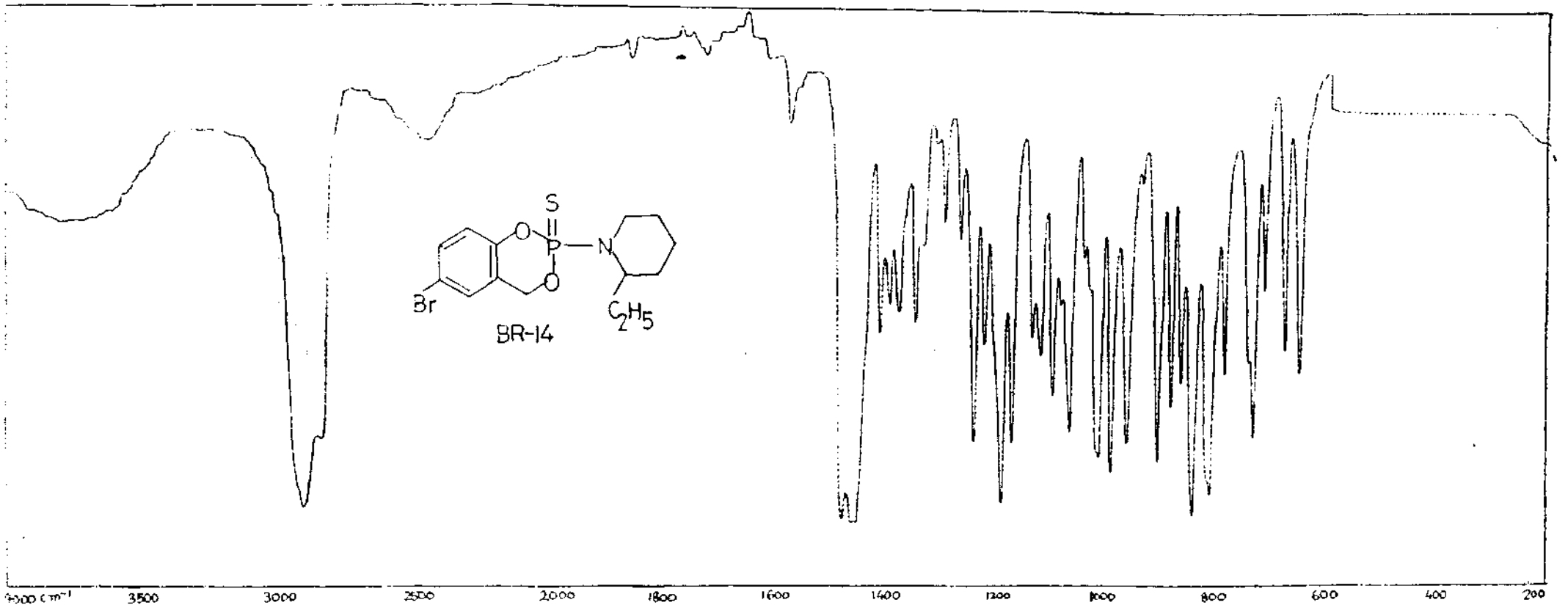


Fig 2.25: IR spectrum of 2-(2-Ethylpiperidino)-6-bromo-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BR-14).

MASS SPECTRUM : (3)
SAMPLE:BR-14
NOTE :DR.B.K.DAS,<2/5/88>
R.T. 0:16" TIM 0.0 RIC 698.0
BASE PEAK : M/E 340.0 INT. 72.6

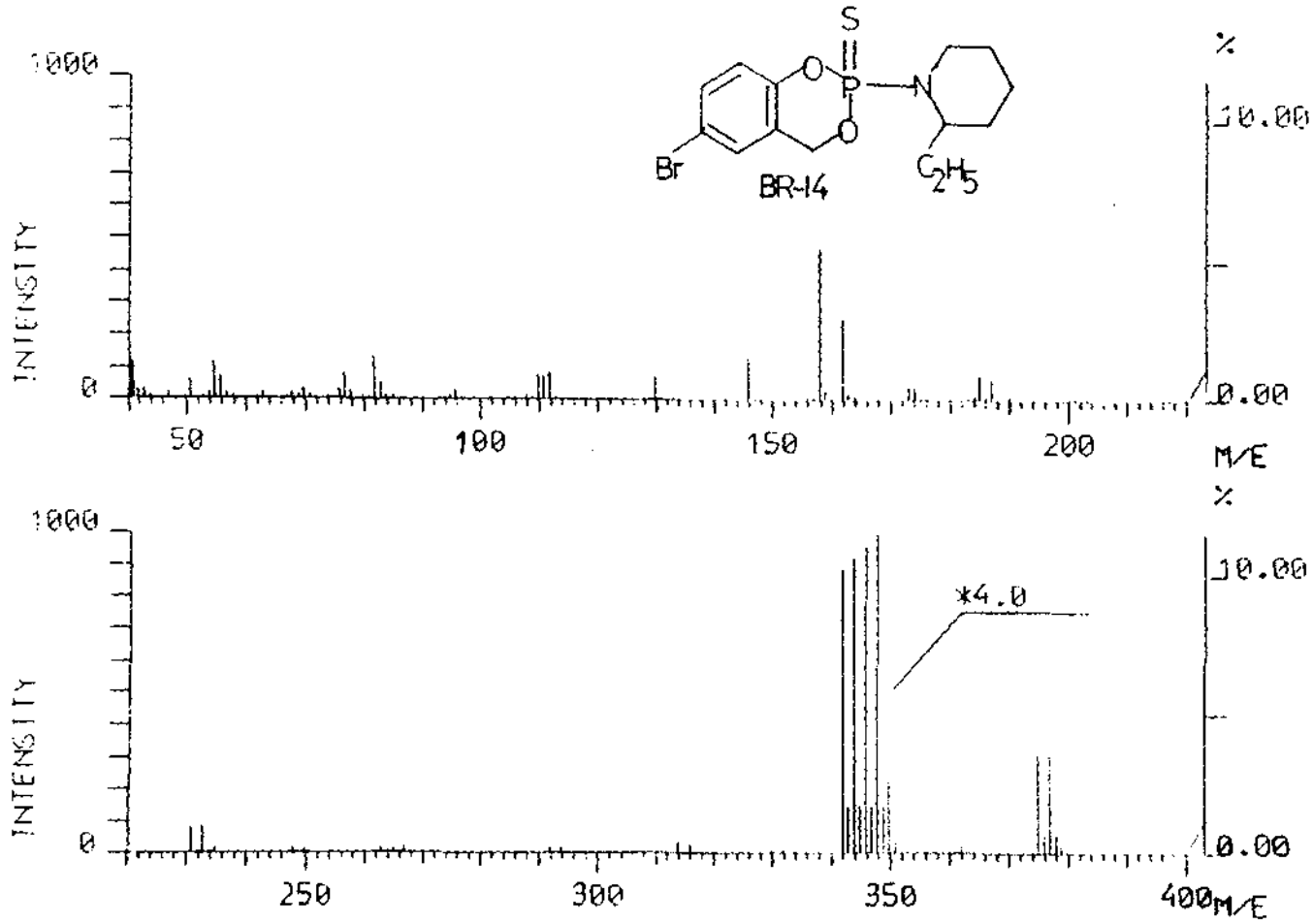


Fig.2.26: Mass spectrum of 2-(2-Ethylpiperidino)-6-bromo-4H-1,3,2-benzodioxaphospnorin 2-sulphide (BR-14).

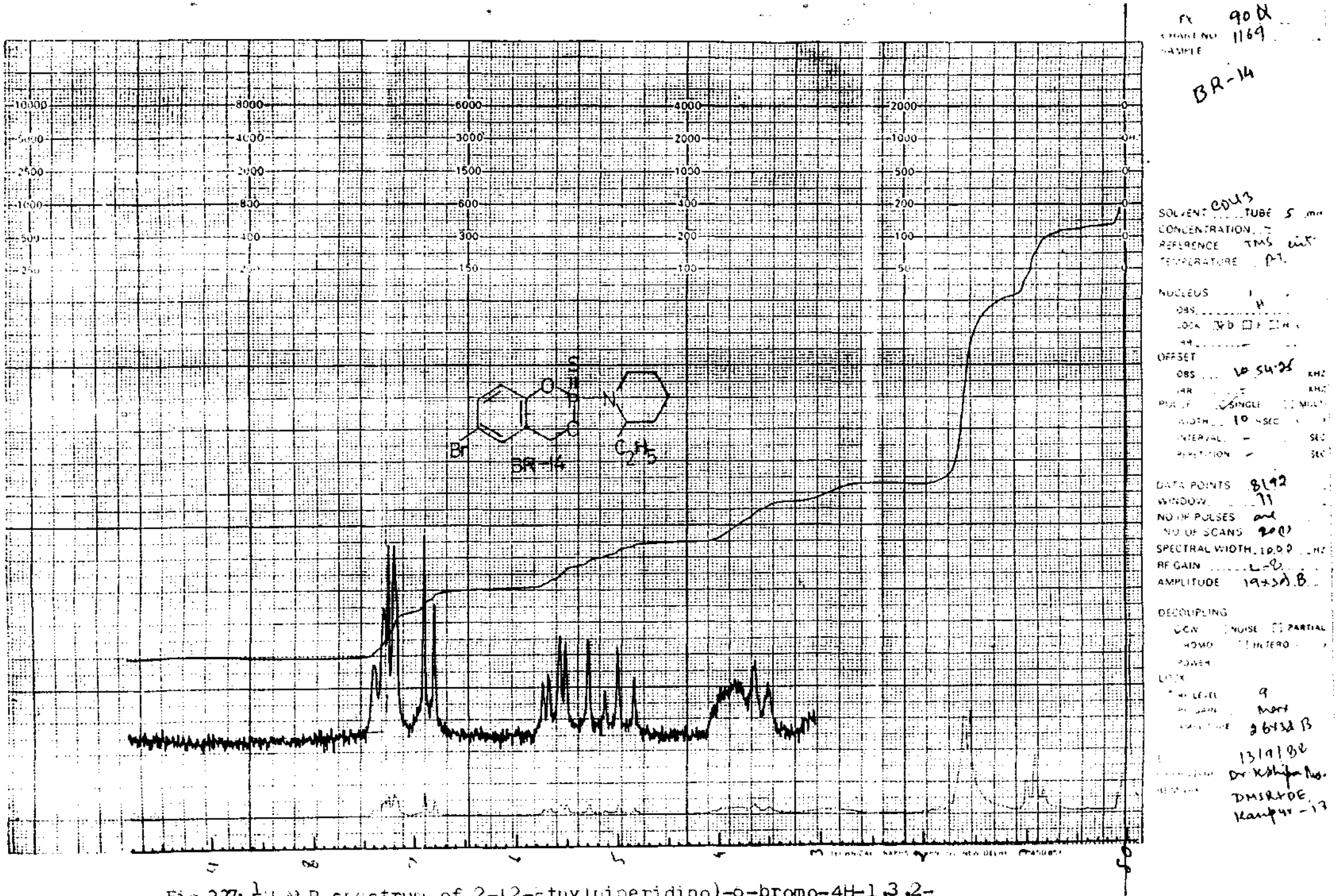
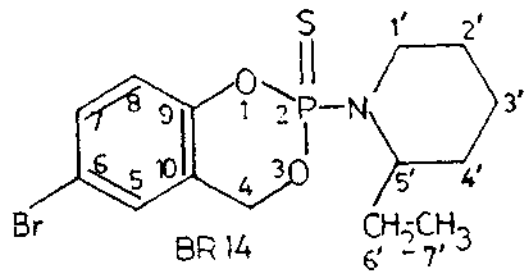


Fig 2.27: ^{1}H NMR spectrum of 2-(2-ethylpiperidino)-o-bromo-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BR-14).



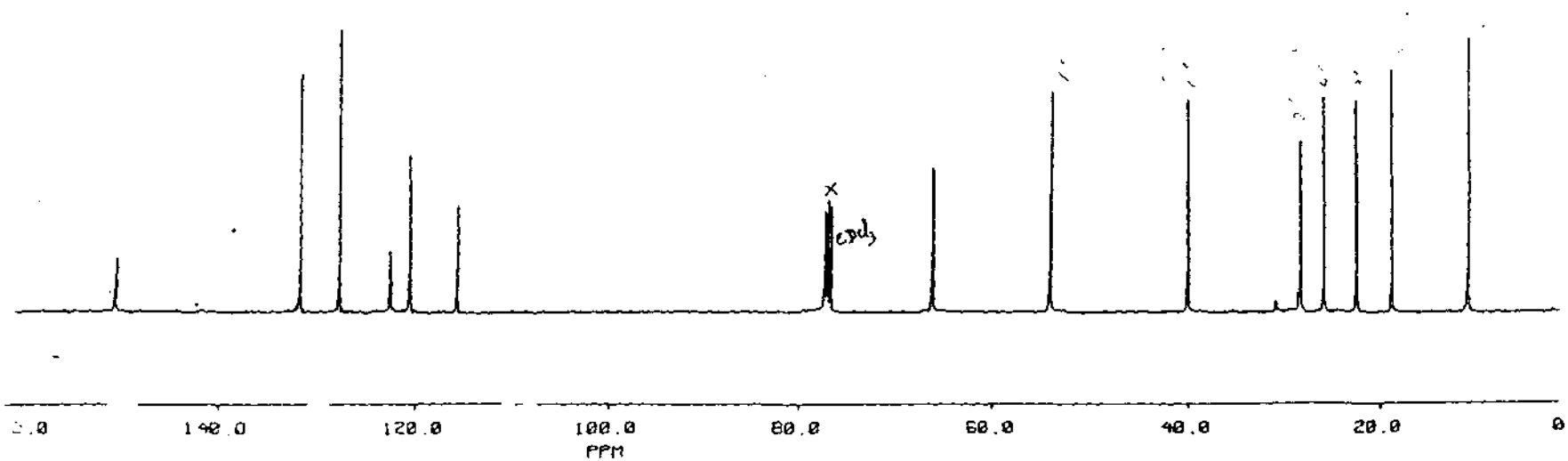


Fig.2.28. 100 MHz proton noise decoupled ^{13}C spectrum of 2-(2-Ethylpiperidino)-6-bromo-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BR-14).

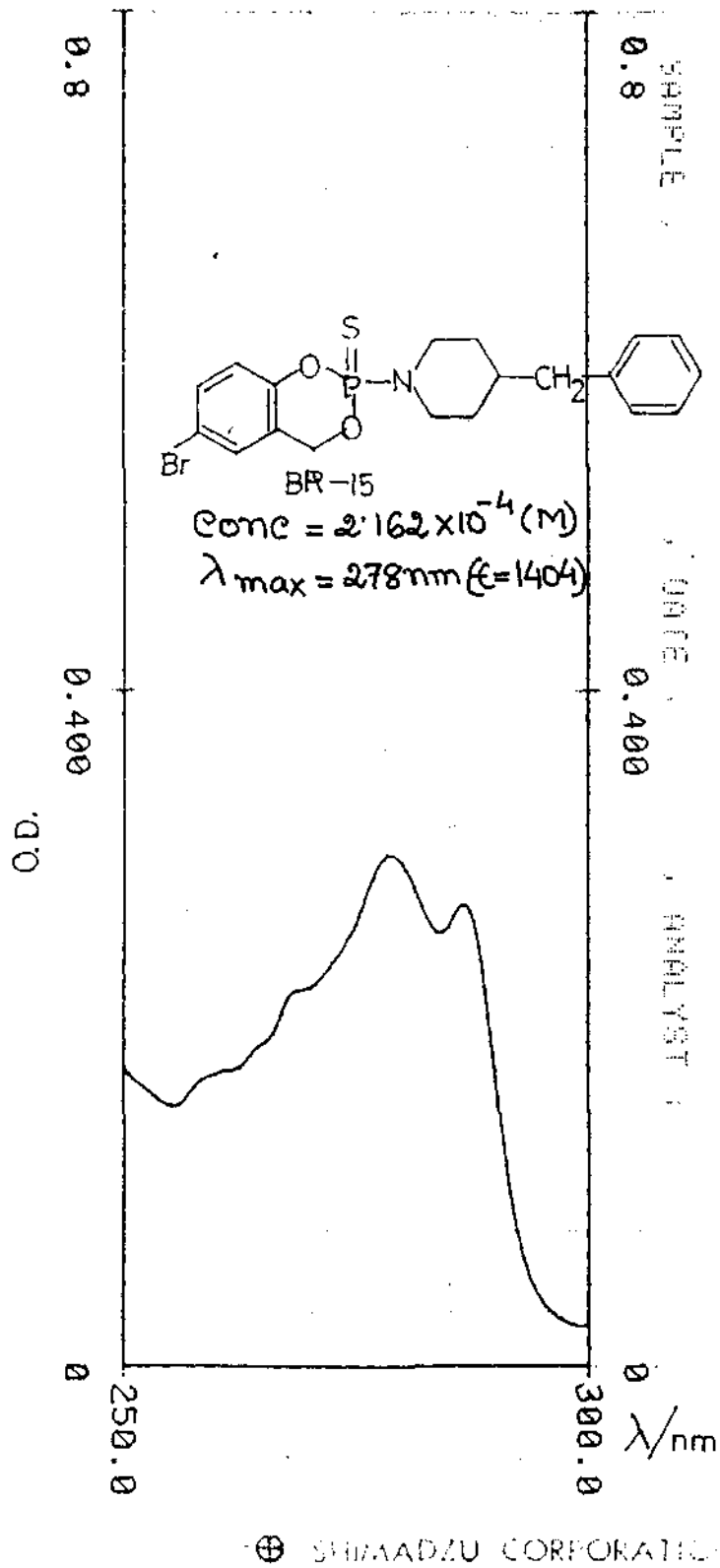


Fig 2.29: Electronic absorption spectrum of 2-(4-Benzylpiperidino)-6-bromo-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BR-15).

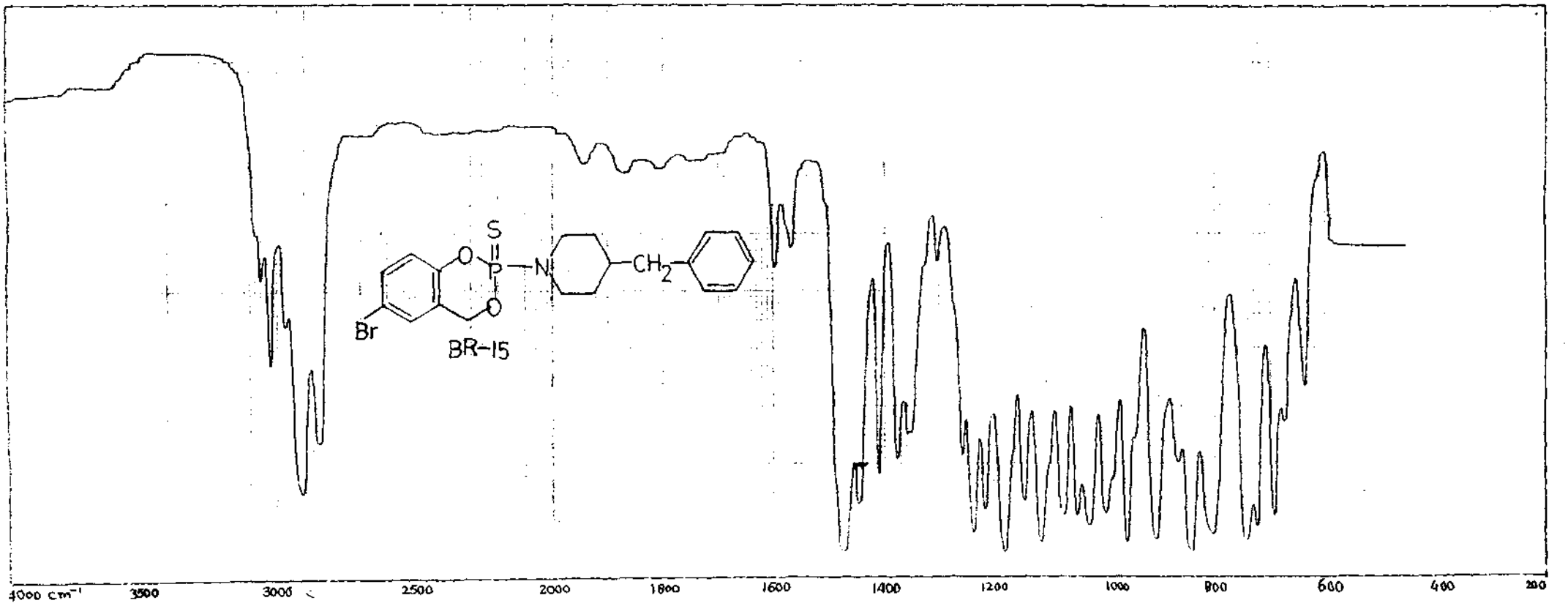


Fig.230: IR spectrum of 2-(4-Benzylpiperidino)-o-bromo-4H-1,3,2-benzodioxaphospnorin 2-sulphide (BR-15).

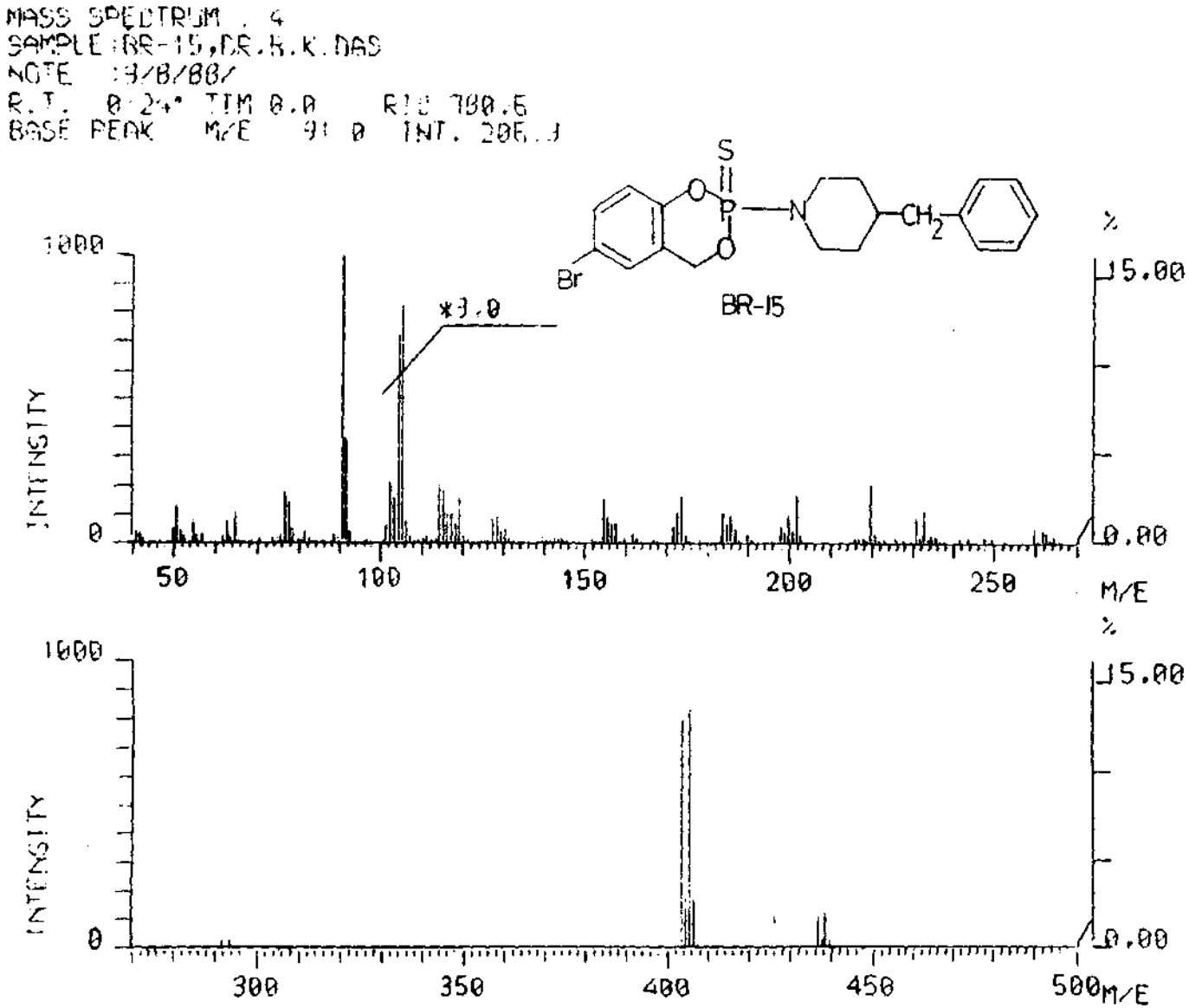


Fig.231: Mass spectrum of 2-(4-Benzyl-piperidino)-6-bromo-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BR-15).

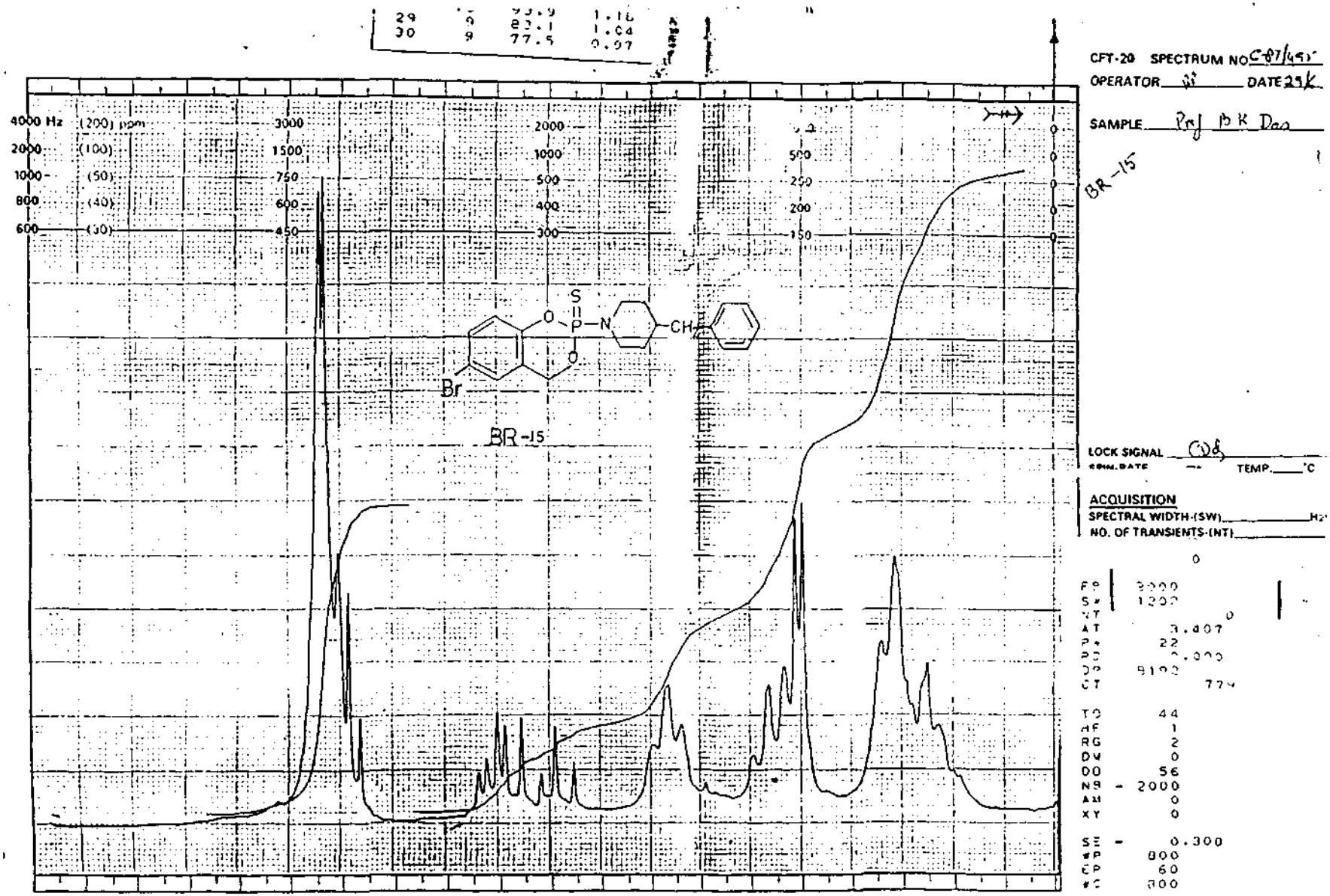


Fig.32: ^1H NMR spectrum of 2-(4-Benzyl-piperidino)-6-bromo-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BR-15).

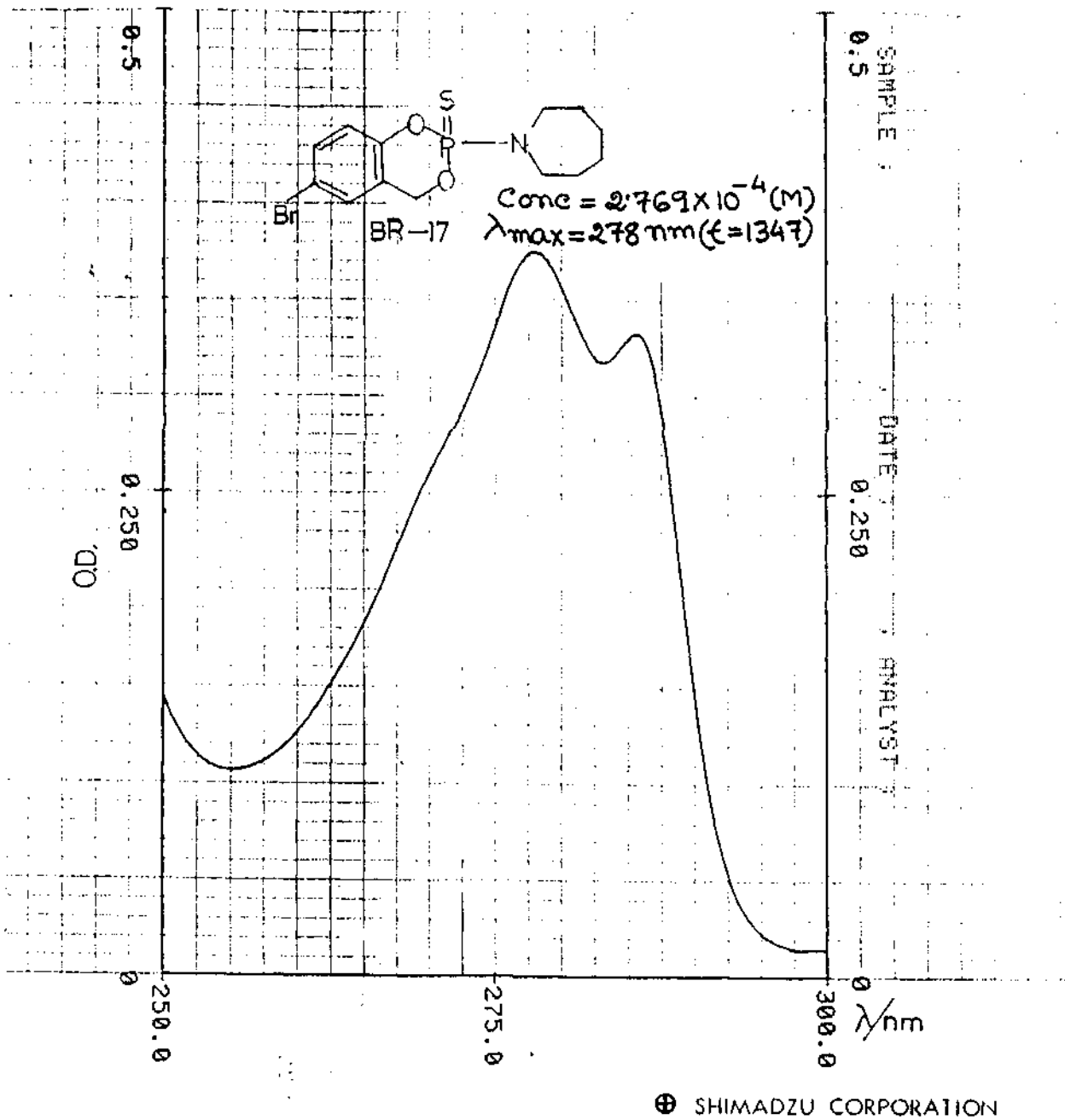


Fig.23: Electronic absorption spectrum of 2-Hexamethylenimido-6-bromo-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BR-17).

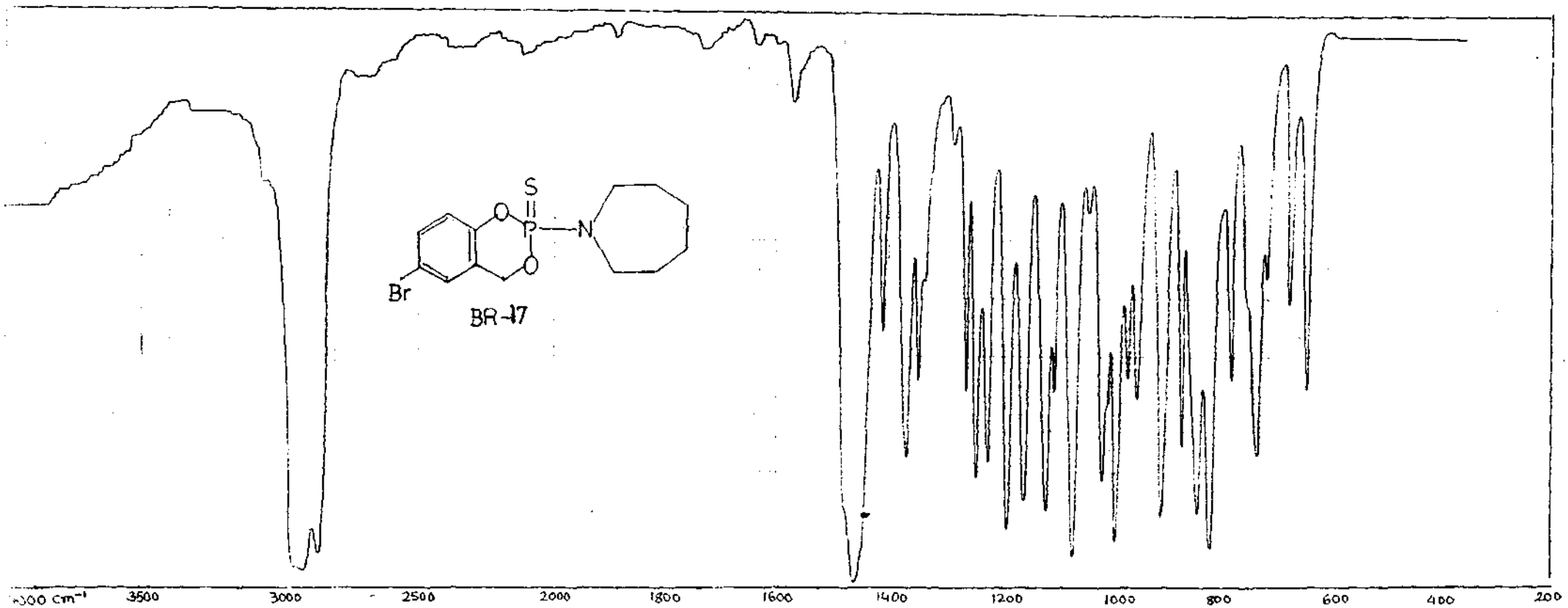


Fig 234: IR spectrum of 2-Hexamethylenimido-6-bromo-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BR-17).

MASS SPECTRUM : (3)
SAMPLE:BR-17
NOTE :DR.B.K.DAS,(2/5/80)
R.T. 0'16" TIM 1.5 RIC 1000.1
BASE PEAK . M/E 328.0 INT. 384.7

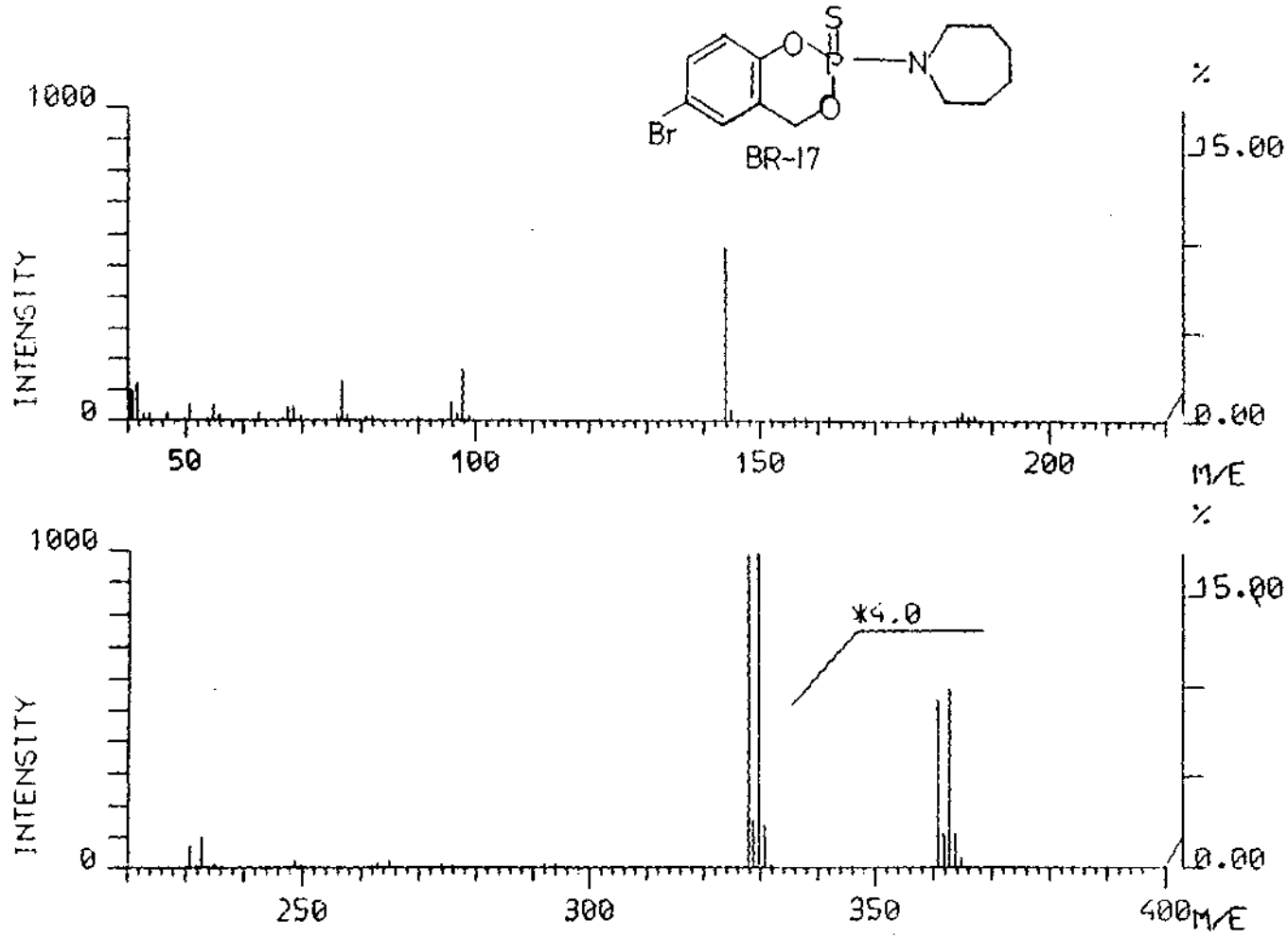


Fig.2.35: Mass spectrum of 2-Hexamethylenimido-6-bromo-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BR-17).

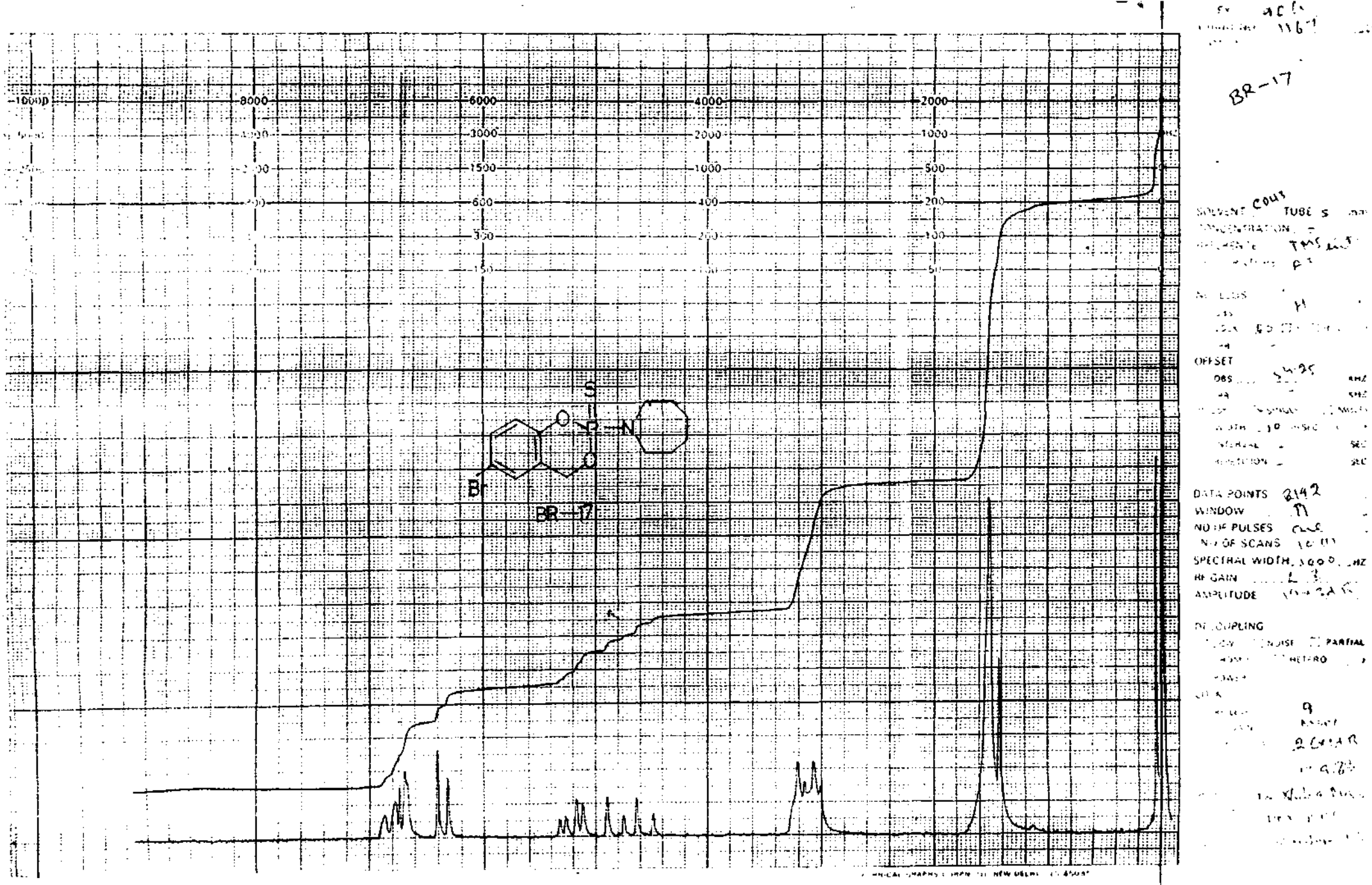
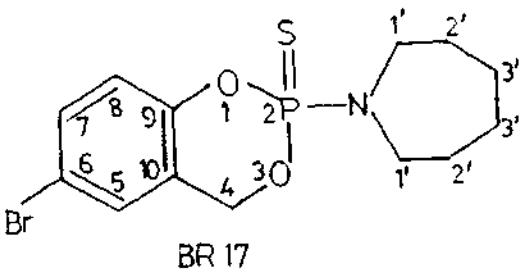


Fig.2.36: ^{1}H NMR spectrum of 2-Hexamethylenimido-6-bromo-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BR-17).



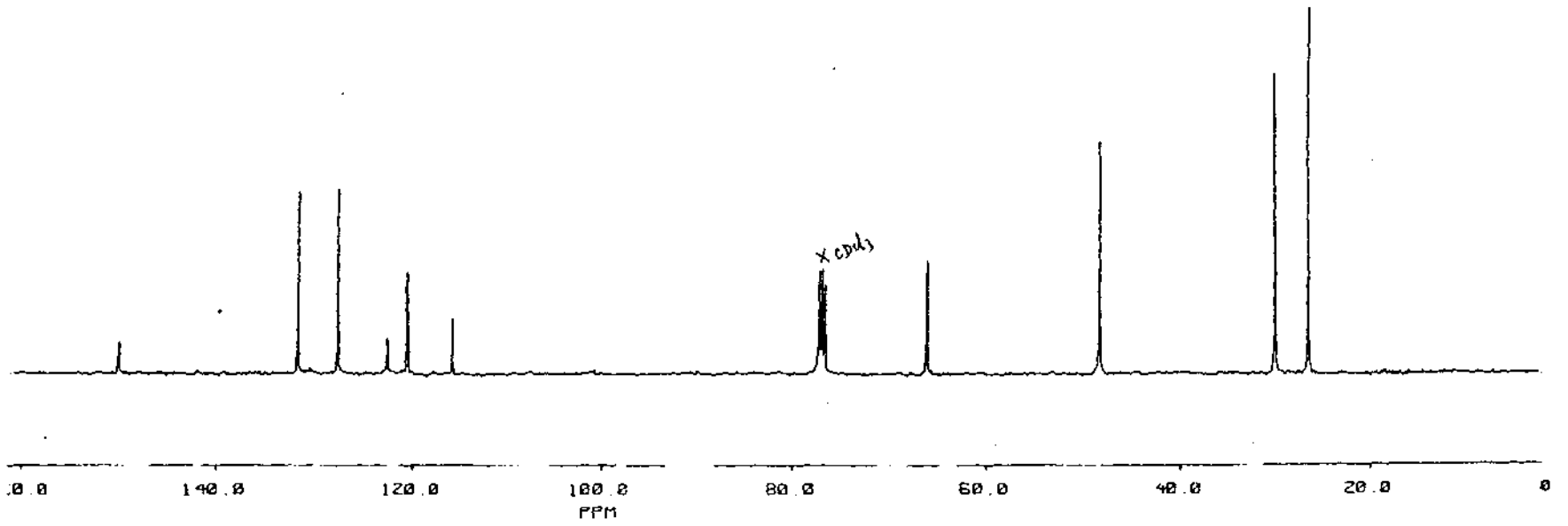
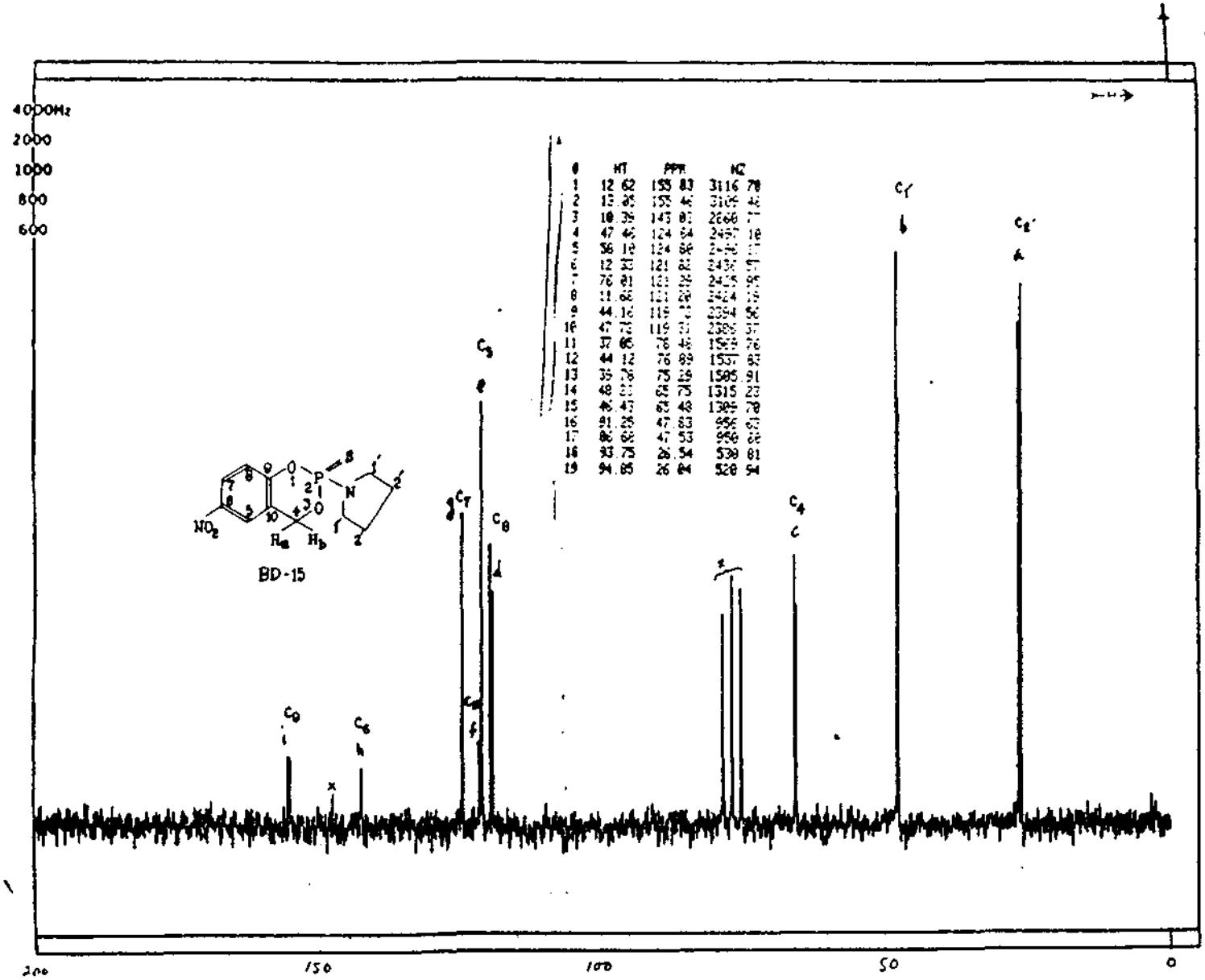


Fig.2.37 . 100 MHz proton noise decoupled ^{13}C spectrum of 2-Hexamethylenimido-6-bromo-4H-1,3,2-benzodioxaphosphorin 2-sulphide (BR-17).



#	HT	PPM	HZ
1	12.62	155.83	3116.78
2	13.05	155.46	3109.42
3	10.39	143.01	2860.77
4	47.46	124.64	2493.10
5	56.10	124.66	2493.37
6	12.33	121.82	2436.57
7	76.01	121.35	2425.95
8	11.66	121.28	2424.19
9	44.16	119.72	2394.56
10	47.73	119.31	2385.37
11	37.86	78.48	1569.76
12	44.12	76.89	1537.83
13	39.76	75.29	1505.91
14	48.21	65.75	1315.23
15	46.47	65.48	1309.78
16	91.25	47.83	956.63
17	86.68	47.53	950.66
18	93.75	26.54	530.81
19	94.85	26.04	520.94

FT-80A SPECTRUM NO 14943-C
OPERATOR
DATE
NUCLEUS
FREQUENCY
SYNTHESIZER SETTING
EXPERIMENT NAME
FILE NAME
SAMPLE BD-15

LOCK ☒ INTERNAL ☐ EXTERNAL
LOCK SIGNAL $CDCl_3$
SPIN RATE 70 rps TEMP 30 C
INSERT 5 mm

ACQUISITION
SPECTRAL WIDTH (SW) 5000 Hz
NO. OF TRANSIENTS (NT) 10,000
ACQUISITION TIME (AT) 0.8 sec
PULSE WIDTH (PW) 3.2 sec
PULSE DELAY (PD) 1 sec
DATA POINTS (DP) 16K

TRANSMITTER OFFSET (TO) 27.07
HIGH FIELD ✓ LOW FIELD
RECEIVER GAIN (RG) 1

DECOUPLER MODE (DM) ✓
DECOUPLER OFFSET (DO) 3.7
NOISE BANDWIDTH (NB) 1.2 kHz
ACQUISITION MODE (AM) 0

DISPLAY
SENS ENHANCEMENT (SE) 0 -2.0 sec
WIDTH OF PLOT (WP) 5000 Hz
END OF PLOT (EP) 0 Hz
WIDTH OF CHART (WC) 5000 Hz
END OF CHART (EC) 0 Hz
VERTICAL SCALE (VS) 100
REFERENCE LINE (RL) 5680.5 Hz

200 MHz proton noise · decoupled ^{13}C spectrum of BD-15 in $CDCl_3$

Fig. 2.38

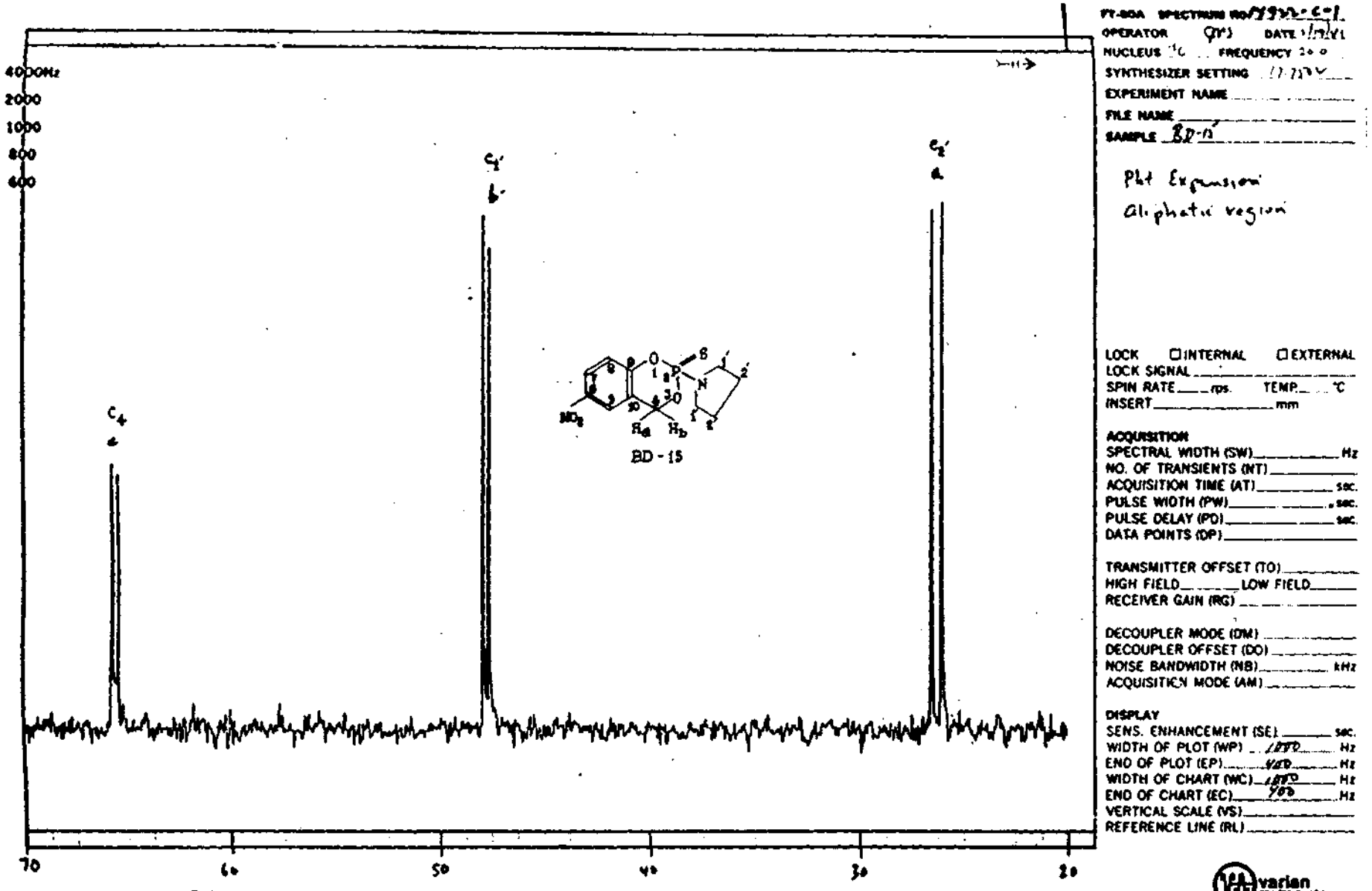


Fig.2.39 : ^{13}C NMR Spectrum of BD-15 (Plot expansion : Aliphatic region).

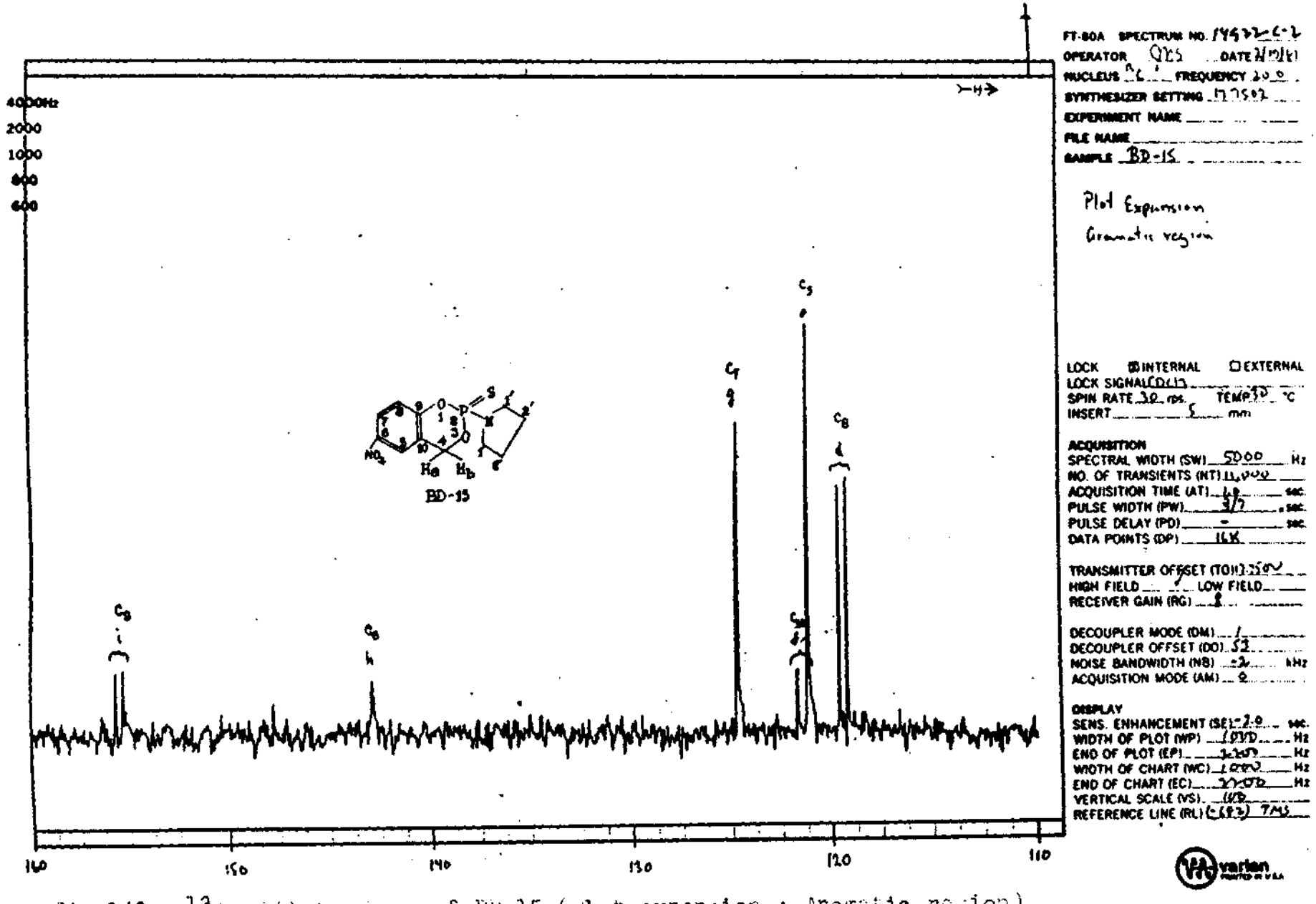


Fig.2.40 : ^{13}C NMR Spectrum of BD-15 (Plot expansion : Aromatic region).

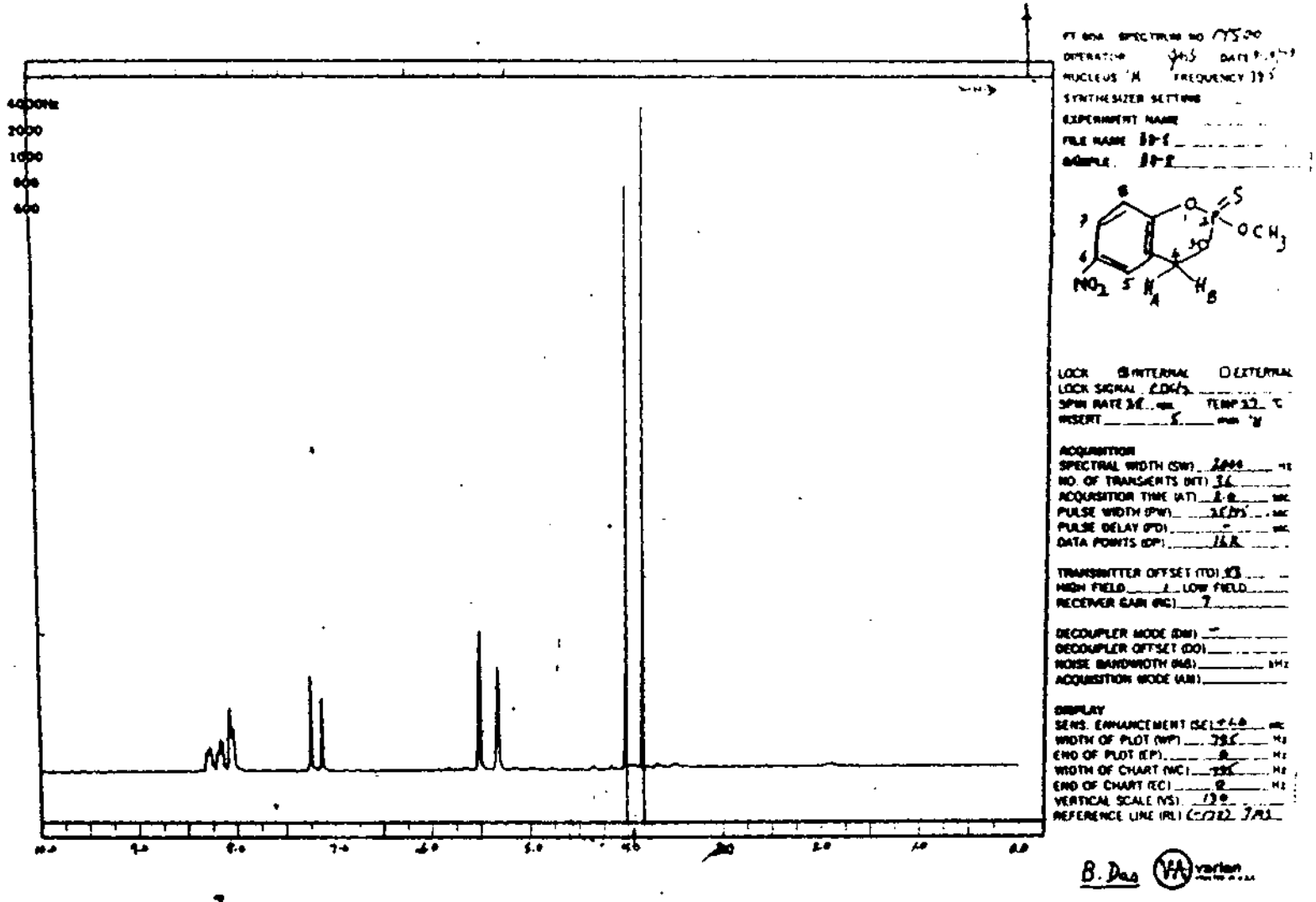


Fig.34: ^{1}H NMR spectrum of 2-Methoxy-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-sulphide (BD-8).

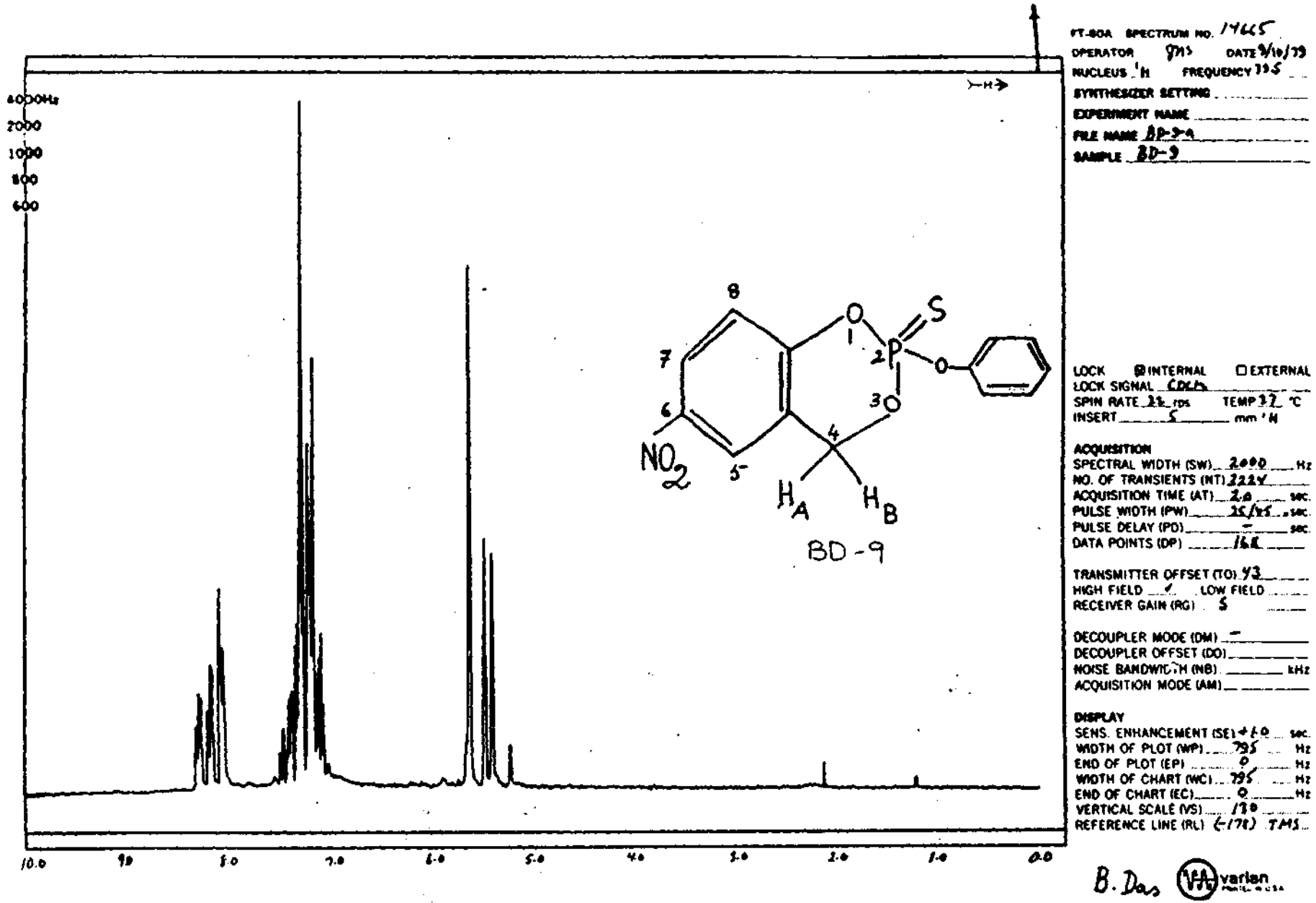


Fig.2.42: ^{1}H NMR Spectrum of 2 - Phenoxy - 6 - nitro - 4H - 1,3,2-benzodioxaphosphorin 2 - sulphide (BD-9).

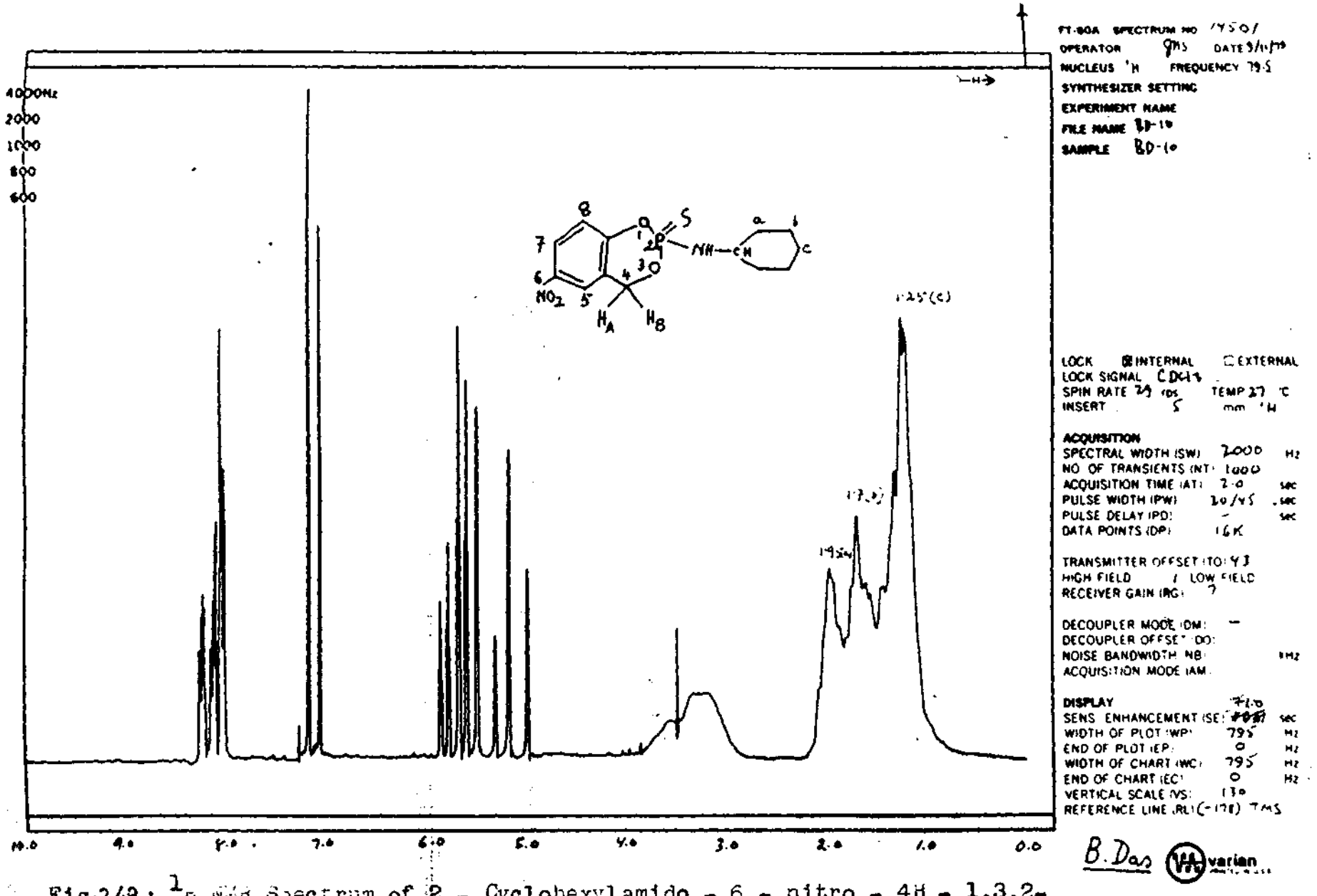
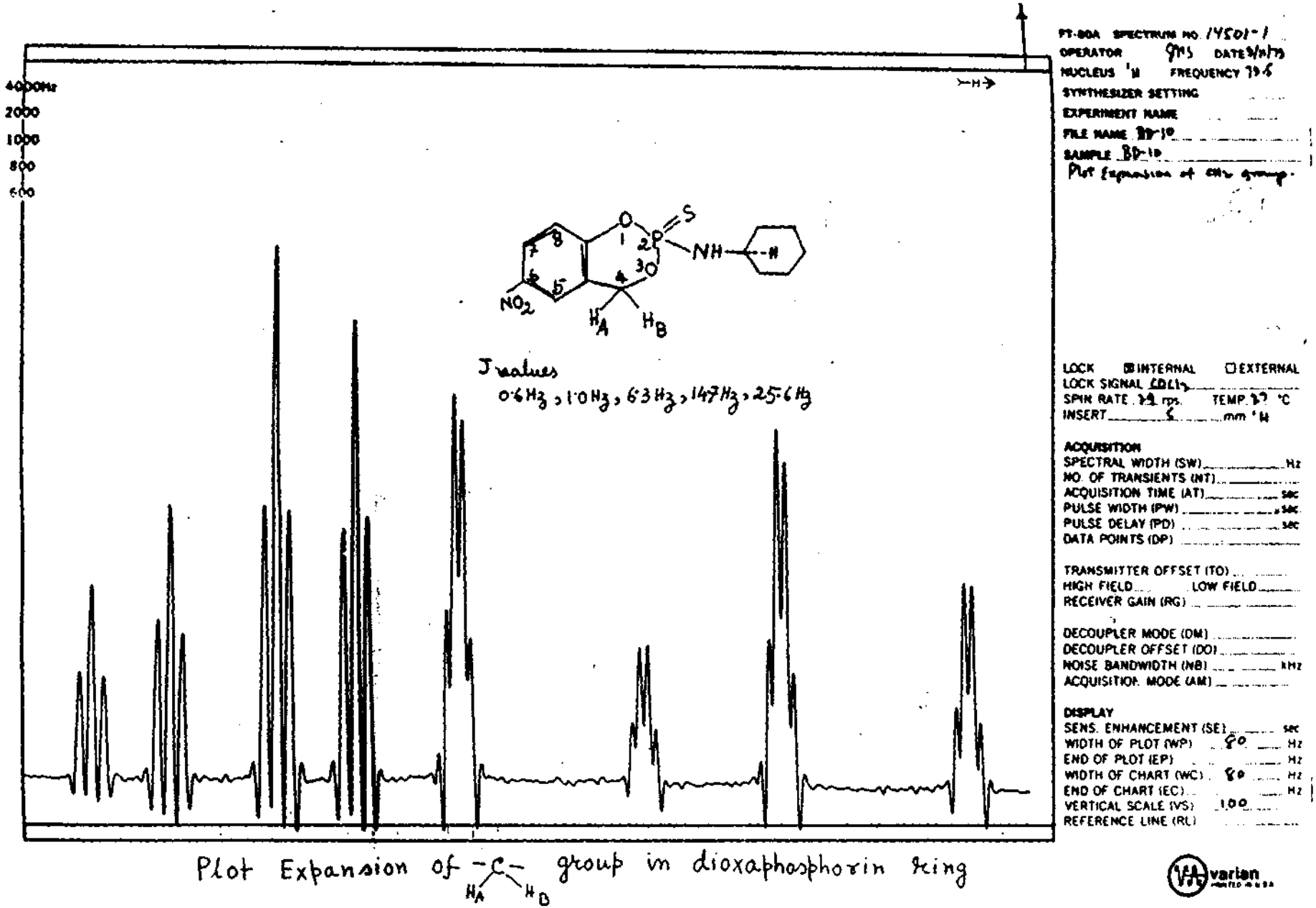


Fig.243 : ^{1}H NMR Spectrum of 2 - Cyclohexylamido - 6 - nitro - 4H - 1,3,2-benzodioxaphosphorin 2 - sulphide (BD-10).



Plot Expansion of $-\underset{H_A}{\overset{H_B}{C}}-$ group in dioxaphosphorin ring

Fig. 2.44 : Plot expansion of the $-CH_2-$ group in BD-10 (5.0 - 5.8 ppm region).

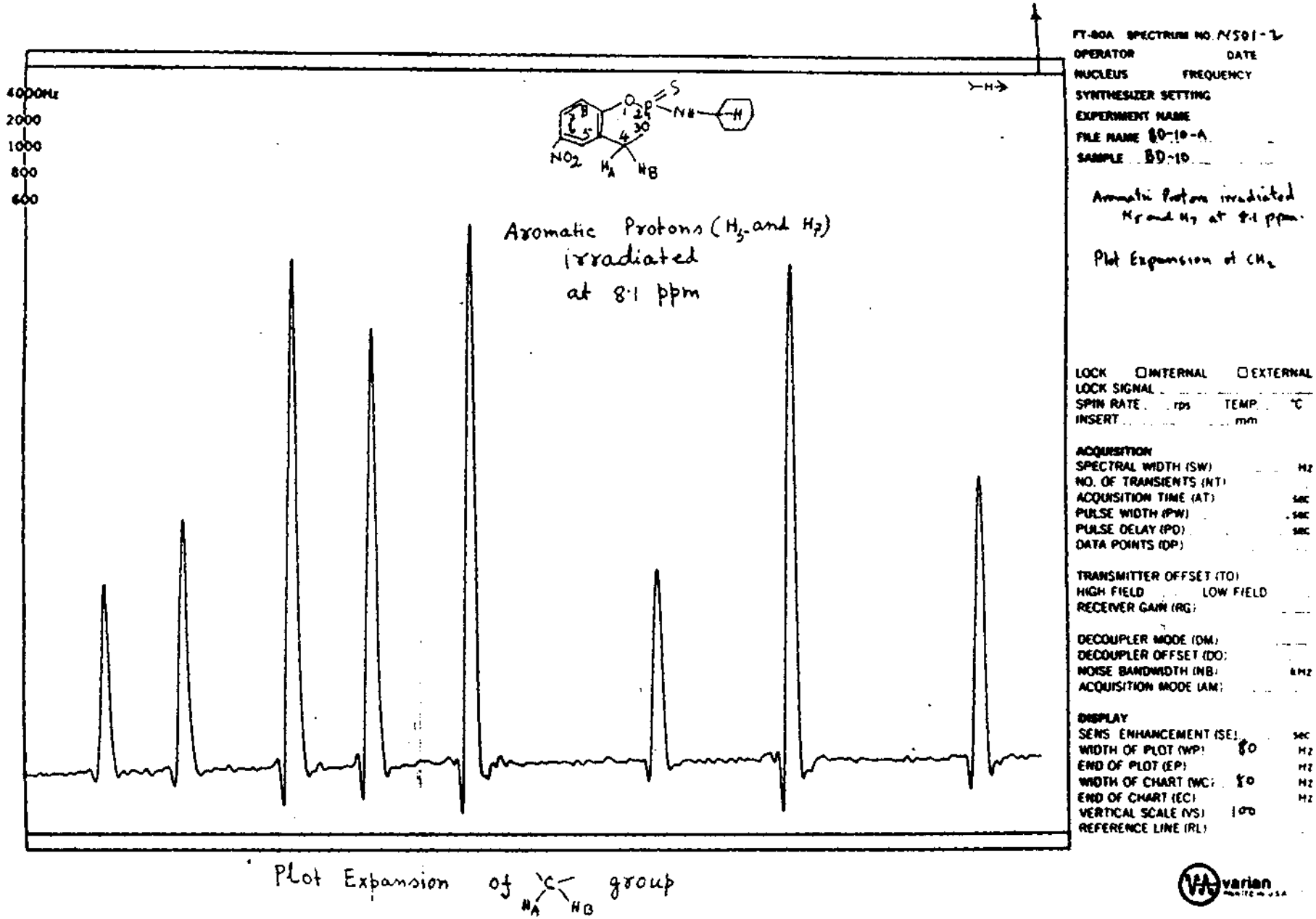


Fig.2.45 : Plot expansion of $-CH_2-$ group in BD-10. (aromatic protons H_5 and H_7 irradiated at 8.1 ppm).

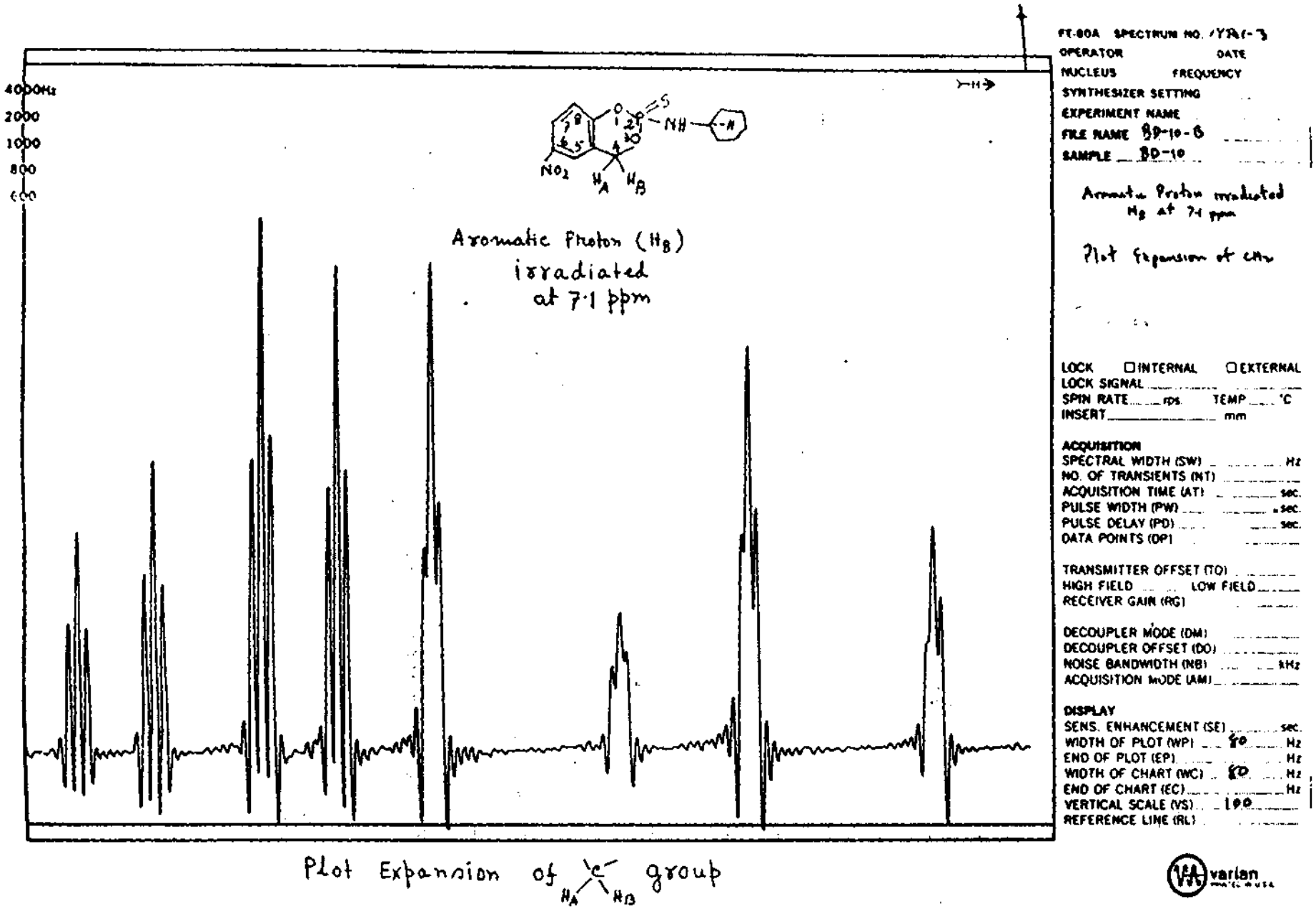
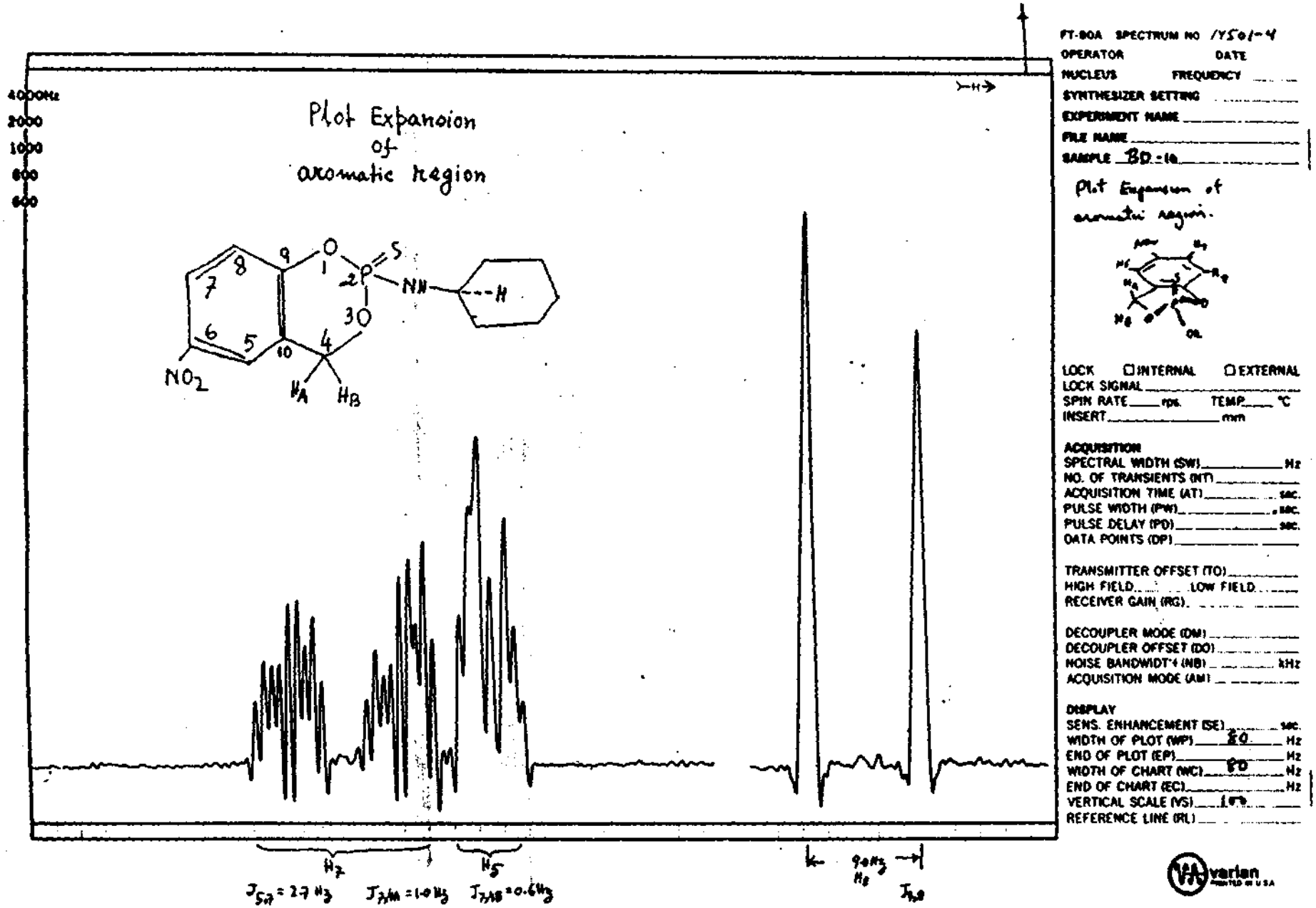


Fig. 2.46 : Plot expansion of $-CH_2-$ group in BD-10 (aromatic proton H_8 irradiated at 7.1 ppm.)



Fir.247: Plot expansion of aromatic region of BD-10.

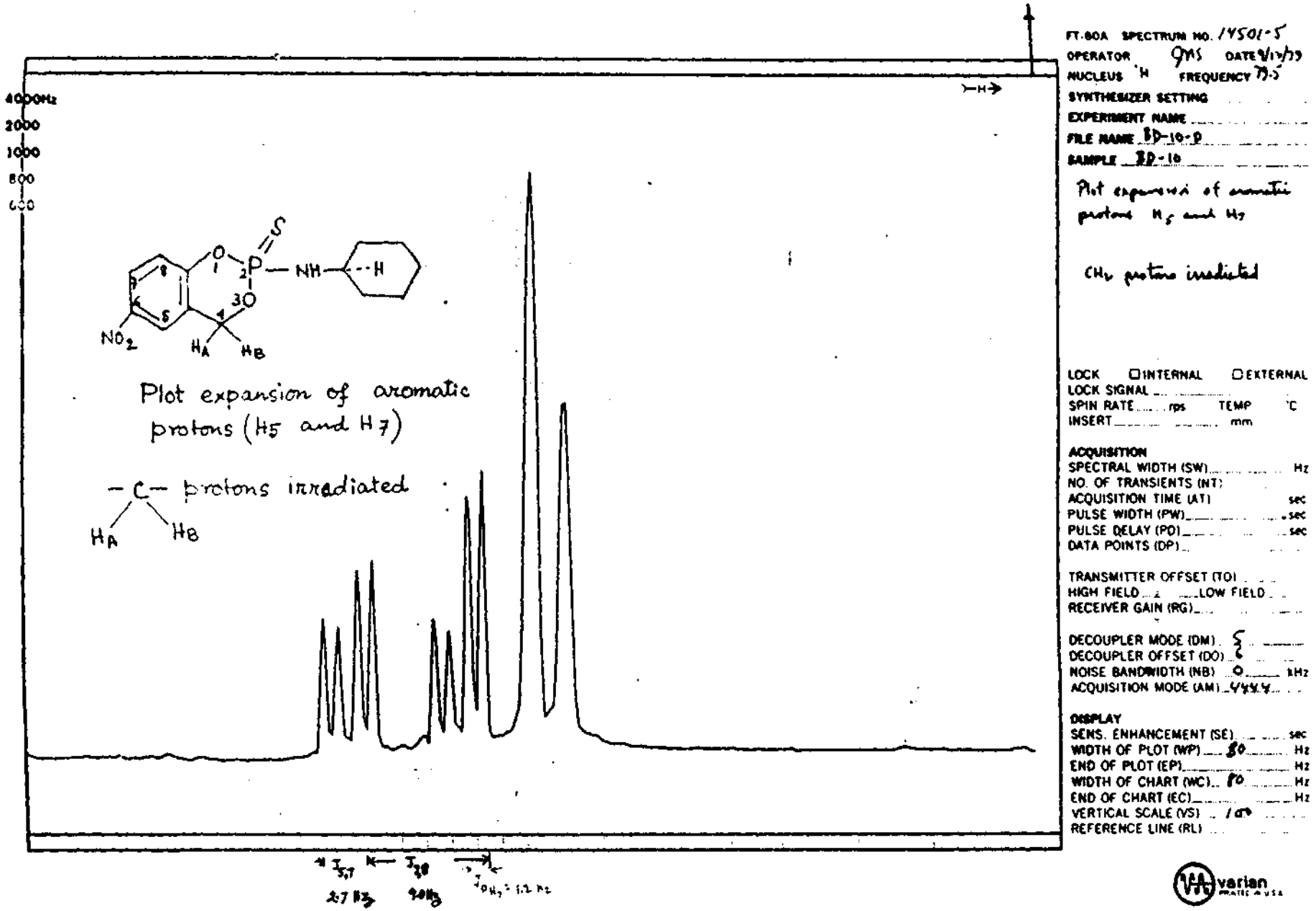


Fig.2.48: Plot expansion of the aromatic protons H_5 and H_7 in BD-10 with the $-CH_2-$ protons (in the dioxaphosphorin ring) irradiated.

APPENDIX - I

Appendix -I

COMMON OR TRADE NAMES, CHEMICAL DESIGNATIONS AND OTHER PROPERTIES OF SOME ORGANOPHOSPHOROUS PESTICIDES.

CONTACT INSECTICIDES

Common or trade name, Chemical name and Chemical designations.	Physical and biological properties.
A. PHOSPHATES	
1. Dichloroves, DDVP, Vapona (R), Nuvan (Shell Oil Co.) $(CH_3O)_2\ P(O)\text{-}OCH = CCl_2$ O,O-dimethyl O-(2,2 dichlorovinyl) phosphate.	Liquid; non-systemic, contact and stomach insecticide with fumigant action and low residual activity; has also acaricidal and anthelmintic property; LD_{50}, 80 mg/kg.
2. Dibrom (R), Naled (California Spray Chemical Corporation, 1956) $(CH_3O)_2\ \overset{O}{\overset{\|}{P}}\text{-}OCH\ Br.\ CBrCl_2$ O,O-dimethyl O-(1,2-dibromo-2,2-dichloro ethyl) phosphate.	Liquid (m.p. of pure compound is 26°C); Non-systemic insecticide and acaricide; LD_{50}, 430 mg/kg.

Contd.....

(Contd.)

3. $(CH_3O)_2\overset{O}{\overset{\|}{P}} - OCH = CHCl$

O,O-dimethyl O-(2-chlorovinyl) phosphate.

Liquid; insecticide; LD_{50}, 50 mg/kg.

4. $(CH_3O)_2\overset{O}{\overset{\|}{P}} - OCH = CClF$

O,O-dimethyl O-(2,2-Chlorofluorovinyl) phosphate.

Liquid; low toxicity to fish.

5. $(C_2H_5O)_2\overset{O}{\overset{\|}{P}} - OCH = CHCl$

O,O-diethyl O-(2-chlorovinyl) phosphate.

Liquid; relatively stable systemic insecticide; LD_{50}, 7.4 mg/kg.

6. Forestenon

$(C_2H_5O)_2\overset{O}{\overset{\|}{P}} - OC(OCH_2CH_2Cl) = CCl_2$

Insecticide ; LD_{50}, 7-10 mg/kg.

7. CH_3O, $ClCH_2CH_2O$, $\overset{O}{\overset{\|}{P}} - OCH = CCl_2$

O-methyl O-(2-chloroethyl) O-(2,2-dichlorovinyl) phosphate.

Systemic insecticide.

(Contd.)

8. (a) SD - 8280

Methyl homolog of chlorfenvinphos

(b) SD - 8211

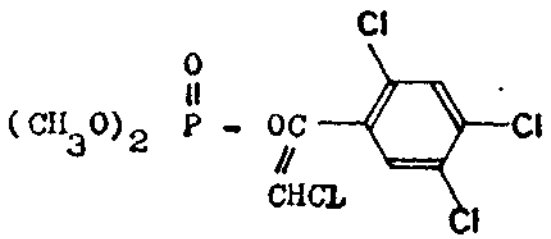
2', 5' - dichloro isomer of methyl homolog of chlorfenvinphos.

Liquid; low toxic insecticide, effective to control flies on faces of hens by feeding.

(SD - 8280 & SD - 8211 have same insecticidal and toxicological properties).

9. Tetrachlorvinphos, Gardona(R), Rabon(R)

(Shell Development CO 1966).



0,0 - dimethyl 0 - [2 - Chloro-1-(2',4',5' - trichlorophenyl) vinyl] phosphate

Solid, mp. 62°C (-isomer) 98°C (-isomer); non-systemic, brief persistence selective insecticide, used to control diptera, lepidoptera and Coleoptera and other pests;

LD_{50} , 4,000 mg/kg.

10. Bonyl(R)

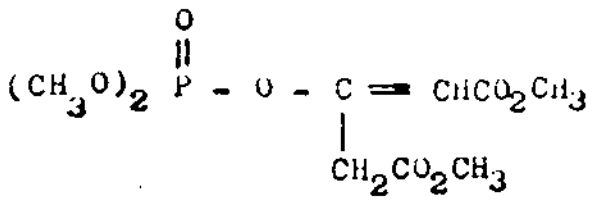
(Allied Chemical Corp., Code No - GC - 3707 1959)

1,3-di(methoxy carbonyl)-1-propen-2-yl dimethyl phosphate

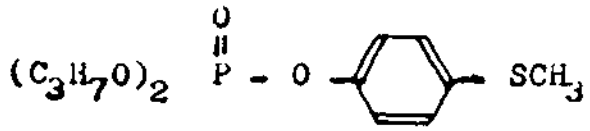
Liquid; used for contact-residual treatment of insects and mites;

LD_{50}, 32 mg/kg (R).

(Contd.)

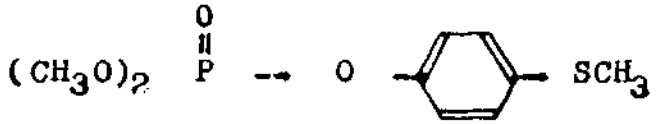


11. (a) Kayaphos(R), Propaphos (Nippon Kayaku Co. 1968)



Liquid; selective contact insecticide for rice stem-borers and green rice leafhoppers; synergists of other organophosphorus insecticide LD_{50}, 70 mg/kg.

(b) GC - 6506

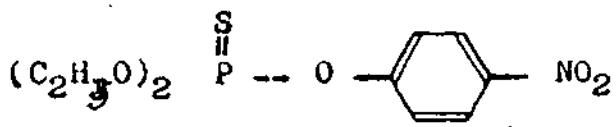


Dimethyl homolog of Propaphos

Insecticide and acaricide for use in cotton; LD_{50} 7 mg/kg. Diethyl homolog is most toxic to mammals.

B. <u>PHOSPHOROTHIONATES</u>.

12. Parathion, Folidol(R), (Bayer AG 1944)

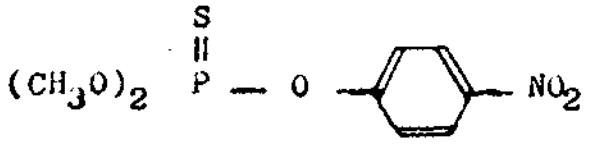


Liquid; wide spectrum insecticide LD_{50}, 7 mg/kg.

O,O-diethyl O-paranitrophenyl phosphorothionate.

(Contd.)

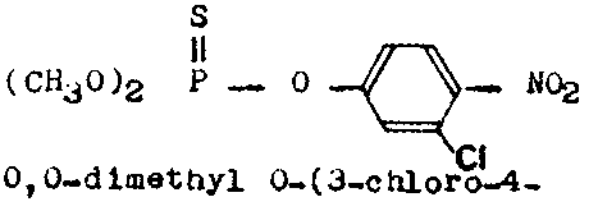
13. Methyl parathion (Bayer AG 1949)



O,O-dimethyl O-paranitrophenyl

Liquid; broad-spectrum; LD_{50}, 25-50 mg/kg.

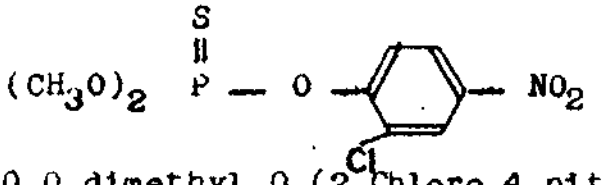
14. Chlorothion(R) (Bayer AG 1952)



O,O-dimethyl O-(3-chloro-4-nitrophenyl).

Crystalline powder; for control of beetles, apphids, caterpillars LD_{50} 880 mg/kg.

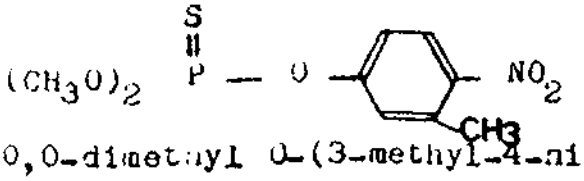
15. Di - Captan(R), Dicapthon (American Cyanamid Co. 1954)



O,O-dimethyl O-(2-Chloro-4-nitrophenyl) phosphorothionate.

Crystalline powder; for controlling household insects, aphids, and boll weevils.

6. Sumithion(R), Folithion(R), Fenitrothion (Sumitome Chemical Co. 1959)

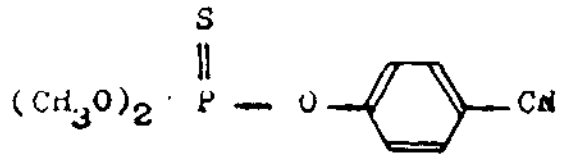


O,O-dimethyl O-(3-methyl-4-nitrophenyl) Phosphorothionate.

Liquid; broad-spectrum (as methyl parathion), specially for rice - stem borers, flies and mosquitoes in public health programmes LD_{50}, 250-500 mg/kg.

(Contd.)

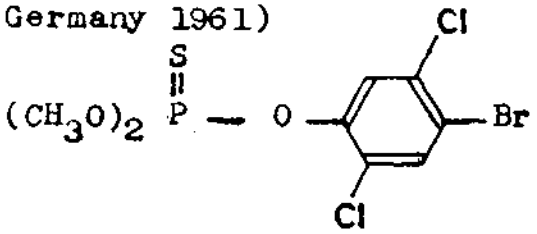
17. Cyanox(R), Cyanophos, CYAP (Sumitomo Chemical Co. 1966)



0,0-dimethyl 0-paracyanophenyl Phosphorothionate.

Liquid; effective against rice-stem borers and also useful for pests of vegetables, fruits, and ornamentals.

18. (a) Bromophos, Nexion(R), Brofene (Boehringer Sohn and Cela Landwirtschaftliche Chemi, Germany 1961)



0,0-dimethyl 0-(4-bromo-2,5-dichlorophenyl) phosphorothionate.

Crystalline solid; mp-54°C; non-systemic persistent insecticide and acariside, particularly effective against diptera; ectoparasite control in livestock; LD_{50}, 3750 to 6100 mg/kg.

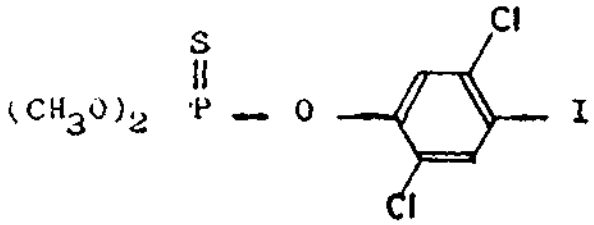
(b) Bromophos - ethyl 0,0-diethyl 0-(4-bromo-2,5-dichlorophenyl) phosphorothionate.

Insecticide and acaricide; LD_{50} 238 mg/kg.

(Contd.)

19. Iodofenphos, Alfacron(R)
Nuvanol N(R), Elocril
(Ciba Ltd. 1966)

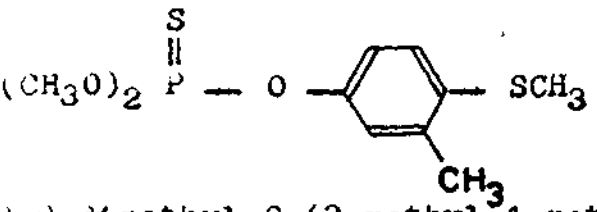
Colourless crystalline solid; mp. 76°C; non- systemic contact and stomach insecticide and acaricide effective against a wide range of insect pests, LD_{50}, 2100 mg/kg.



O,O-dimethyl O-(4-iodo - 2,5-dichlorophenyl) phosphorothionate.

20. Baytex(R), Lebayacid(R),
Fenthion
(Bayer AG. 1958)

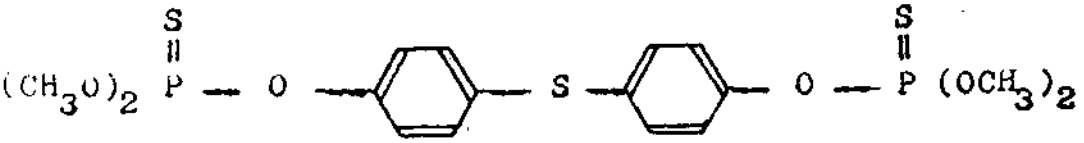
Liquid; general purpose insecticide with systemic action; particularly for fly and mosquito;
LD_{50}, 215 mg/kg (Male R)
615 mg/kg (Female R).



O,O-dimethyl O-(3-methyl-4-methylthiophenyl) phosphorothionate

21. Abate(R), Biothion(R)
(American Cyanamid Co. 1965)

White solid; mp - 30°C; control of mosquito larvae; LD_{50}, 2000-4000mg/kg



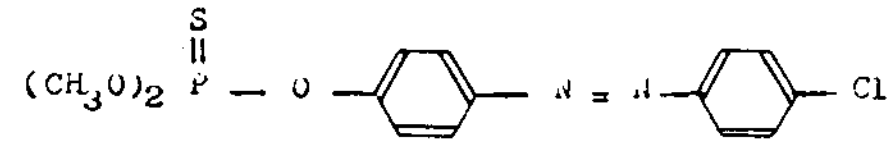
O,O, O',O' - Tetramethyl O,O'-thiodi-p-phenylene phosphorothionate.

(Contd.)

22. Alamos(R), Slam(R), Experimental insecticide and acaricide, particularly nontoxic to mammals.

Azothoate

(Soc. Montecatini, Italy, 1964)

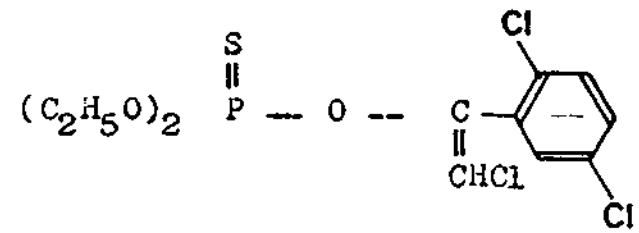


O,O-dimethyl O - [p -(p-chlorophenylazo) phenyl]/phosphorothionate

23. Akton(R) Brown Liquid; non-systemic insecticide, effective particularly for soil insects such as lawn chiuch bugs;

LD_{50}, 146 mg/kg.

(Shell Development Co.)

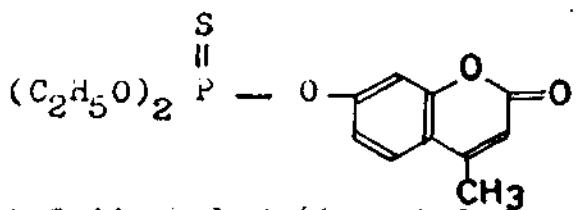


O,O - diethyl O - [2 - chloro-1-(2',5'-dichlorophenyl) vinyl] phosphorothionate

The methyl homolog and 2',4',5' - trichloro-analog of Akton are effective as house fly larvicide.

24. Potasan(R) Colourless crystalline solid, mp. 38°C; stomach insecticide with weak contact activity; particularly effective against Colorado potato beetles, LD_{50}, 42 mg/kg.

(1947)



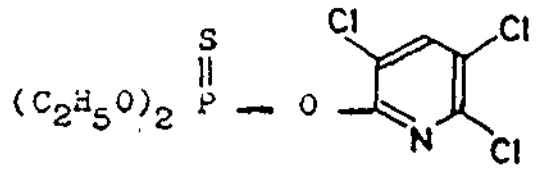
O,O-diethyl O-(4-methyl-7-coumarinyl) phosphorothionate.

(Contd.)

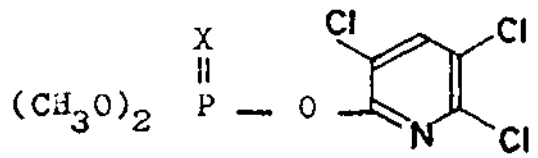
25. Dursban(R),

Chloropyrifos

(Dow Chemical Co. 1965)



O,O-diethyl O-(3,5,6-trichloro-2-pyridyl) phosphorothionate.



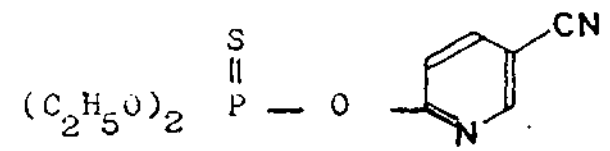
X = S Dowco 214

X = O Dowco 217

The methyl homolog (Dowco(R) 214) is more effective against adult mosquitoes but less effective against the larval than chloropyrifos; its LD_{50} is 1,500 mg/kg.

White crystalline solid, mp. 43°C; moderately persistent insesticide, effective for controlling — mosquito and fly larval, sucking and chewing plant pests, and soil pests, ticks on livestock, LD_{50}, 163 mg/kg.

26.

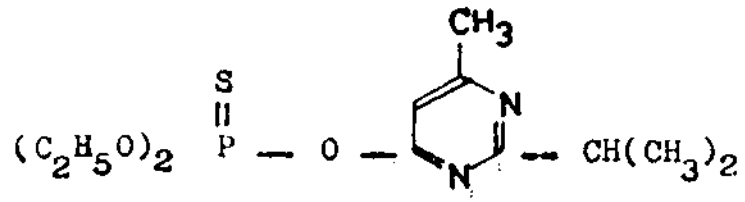


O,O-diethyl O-(5-Cyano-2-pyridyl) phosphorothionate.

Effective against various insects and mites.

(Contd.)

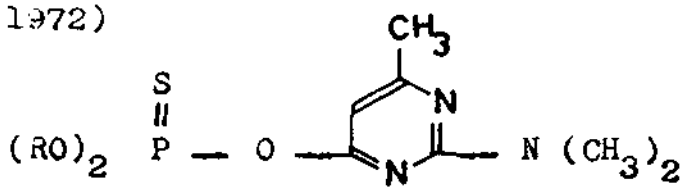
27. Diazinon, Basudin(R), Srolex(R) (1952)



O,O-diethyl O-(2-isopropyl-4-methylpyrimidin-6-yl) phosphorothionate.

Liquid; it has long residual action and effective against soil, fruit, vegetable, and rice insects; LD_{50}, 108-250 mg/kg. The n-propyl isomer of diazinon (Pyrazinon) is also effective as an insecticide; LD_{50}, 261 mg/kg.

28. Primiphos - ethyl (Imperial Chemical Industries 1972)

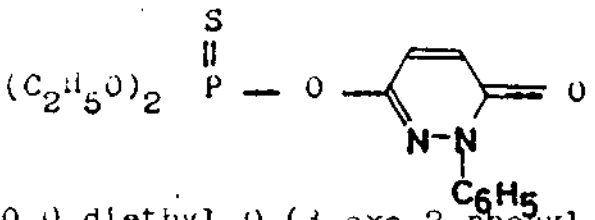


R = C_2H_5 primiphos - ethyl
R = CH_3 primiphos - methyl

O, O-diethyl O-(2-dimethylamino-4-methylpyrimidin-6-yl) phosphorothionate.

Primiphos - ethyl is a liquid, with broad insecticidal spectrum, particularly effective against diptera and colecptera; it has also fungicidal activity; LD_{50}, 140-200 mg/kg; Primiphos-methyl is a liquid; it is an insecticide and acaricide with contact and fumigant activity; it is also used for public health: LD_{50} 2050 mg/kg.

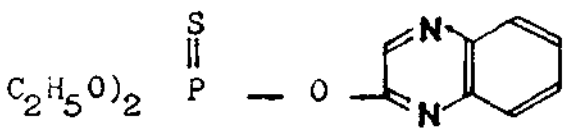
29. Pyridafenthion, Ofnack(R), AC-12, 503 (Mitsui-Toatsu Chemicals. 1973).



O,O-diethyl O-(3-oxo-2-phenyl-2H-pyridazin-6-yl) phosphorothionate.

Used for the control of rice stem borers.

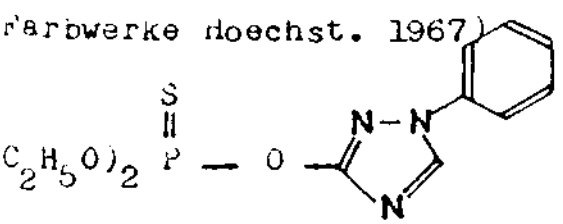
30. Quinalphos, Bayrusil(R) Diethquinalphion. (Bayer AG. 1965)



O,O-diethyl O-(2-quinoxalyl) phosphorothionate.

White crystalline powder, mp - 36°C; contact and stomach activity; effective against both biting and sucking pests, especially against diamond back moth; for control of mosquitoes and mites; LD_{50} 66 mg/kg.

31. Hostathion(R), Triazophos, HOE 2960; (Farbwerke Hoechst. 1967)

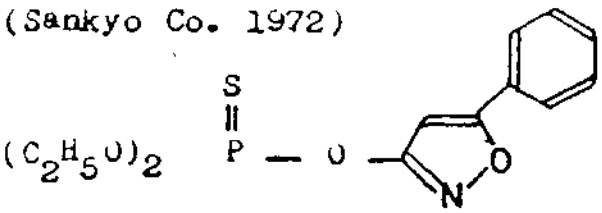


O,O-diethyl O-(1-phenyl-1,2,4-triazol-3-yl) phosphorothionate.

Liquid; a non-systemic insecticide and acaricide, acting as a contact and stomach position, LD_{50}, 82 mg/kg.

(Contd.)

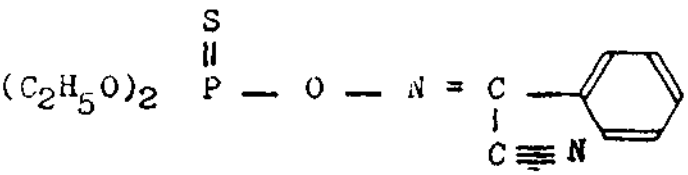
12. Isoxathion, Karphos(R)
(Sankyo Co. 1972)



O,O-diethyl O-(5-phenyl-3-isoxazolyl) phosphorothionate. The dimethyl homolog [Dimex(R), LD_{50}, 593 - 697 mg/kg (M)] is less toxic.

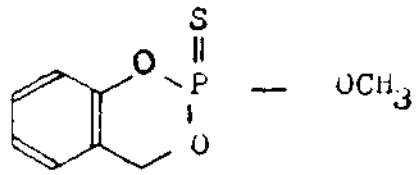
Liquid, a broad spectrum insecticide, including scale insects and soil insects; LD_{50}, 112 mg/kg. The diethyl phosphorothionate ester of non-substituted 3-hydroxy isoxazole is more effective insecticide than isoxathion, but too toxic, LD_{50}, 5-10 mg/kg (M).

13. Phoxim, Baythion(R), Valexon
(Bayer AG. 1965)



Liquid; broad spectrum, contact and stomach poison, most useful against soil insects and stored products pests; LD_{50}, 2000 mg/kg

14. Salithion
(Sumitomo Chemical Co. 1968)

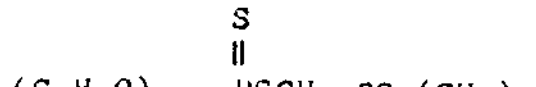


2-methoxy-4H-1,3,2-benzodioxaphosphorin-2-sulphide.

White crystalline powder, m.p. 56°C; a short-lived insecticide for protection of fruits and vegetables; LD_{50}, 102 mg/kg.

Other related compounds:

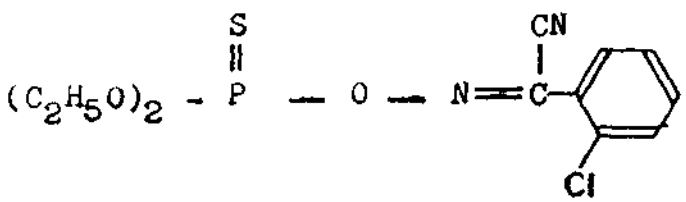
35. Terbufos.



S - tert - butylthiomethyl-
-O, O-diethyl phosphorodithioate.

Colourless to pale yellow liquid, has effective initial and residual activity against soil insects and other arthropods,
LD_{50}, 1.6 mg tech/kg.

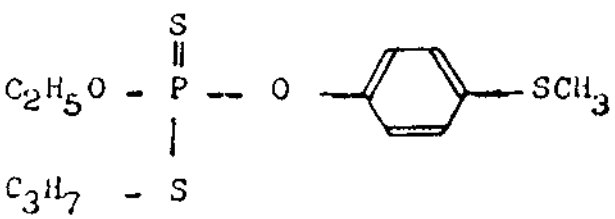
36. Chlorphoxim, Baythion C.
(Bayer AG, 1977)



O,O-diethyl 2-Chloro- -cyano-benzylideneamino-oxyphosphonothionate.

Colourless crystalline solid, m.p. 66.5°, an insecticide used to control mosquitoes and simulium flies,
LD_{50} 2500 mg/kg.

37. Sulprofos, Bolstar, Helothion.
(Bayer AG, 1976)



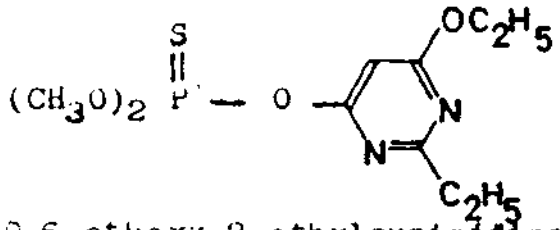
O-ethyl O-(4-methylthiophenyl) S-propyl phosphorodithioate.

Colourless oil, b.p. 210°, has specific effect against Lepidoptera on cotton,
LD_{50}, 304 mg/kg.

(Contd.)

38. Etrimfos, Ekamate.

(Sandoz Ltd., 1972).

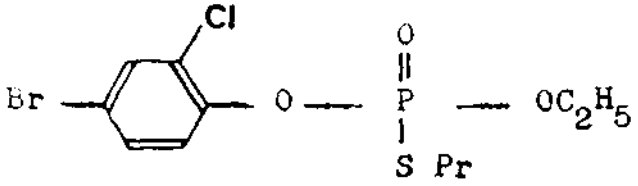


O-6-ethoxy-2-ethylpyrimidine-4-yl- O,O, -dimethyl phosphorothionate.

Colourless oil, broad-range non-systemic insecticide, effective against species of Lepidoptera, Diptera and to a variable extent Hemitera, LD_{50}, 1800 mg/kg.

39. Profenofos, Curacron,

(Ciba - Geigy AG)



O-(4-bromo-2-Chlorophenyl)-O - ethyl S-propyl phosphorothioate.

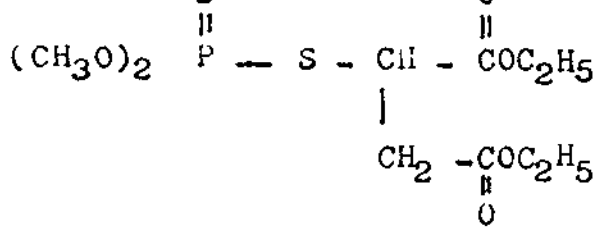
Yellow liquid, non-systemic broad - spectrum insecticide, used against insects, pests and mites on cotton and vegetables,

LD_{50}, 358 mg/kg.

C. PHOSPHOROTHIOLOTHIONATES.

40. Malathion, Cythion(R), Karbophos, (American Cyanamid Co. 1950).

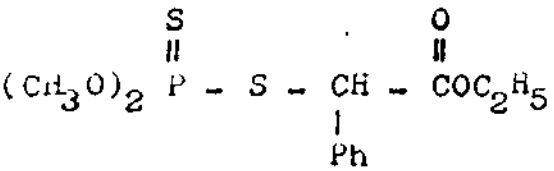
Liquid; safe general purpose insecticide with low mammalian toxicity; LD_{50}, 1375 mg/kg; contact and stomach poison.



O, O-dimethyl S - [1,2-di(ethoxycarbonyl) ethyl] phosphorothiolothionate.

41. Phentboate, Cidial(R), dimephenthoate, Papthion(R), Elsan(R) (Soc. Montecatini, Itali. 1964)

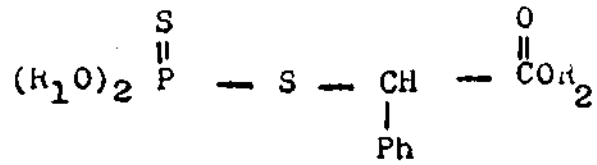
Liquid; broad spectrum insecticide and acaricide; LD_{50}, 4,700 mg/kg.



O,O-dimethyl S - [(α-ethoxycarbonyl) benzyl] phosphorothiolothionate. Some other related compounds have been tested for insecticidal and acaricidal properties.

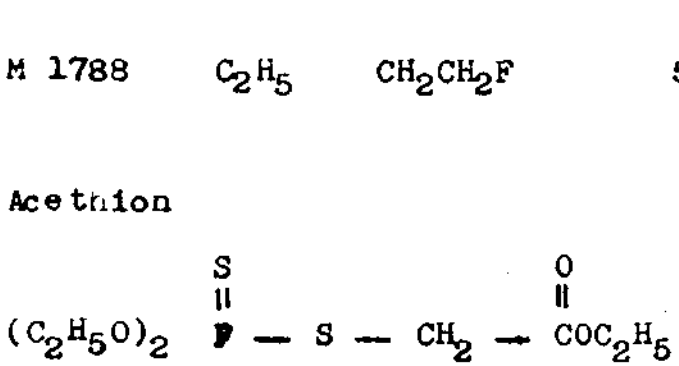
(Contd.)

41. (Contd.....)



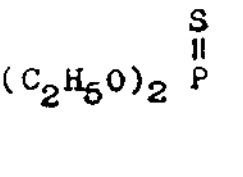
	R_1	R_2	LD_{50} (mg/kg)
M 1703	CH_3	$CH(CH_3)_2$	400 - 500
M 1788	C_2H_5	CH_2CH_2F	5

42. Acethion

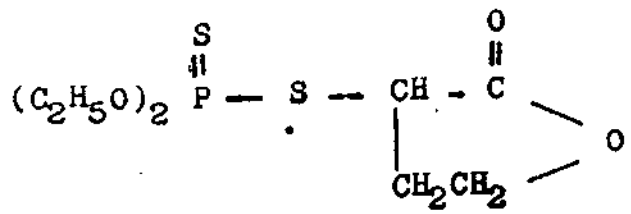


Liquid; selective insecticide; LD_{50}, 1100 mg/kg; it is not used practically.

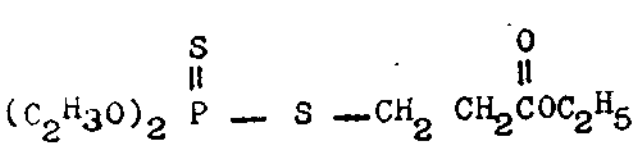
Other related compounds:



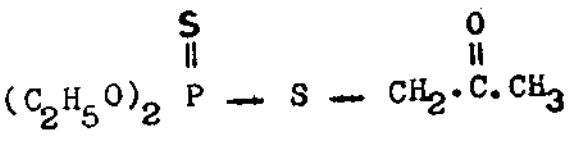
azethion



GC - 9879



Propathion

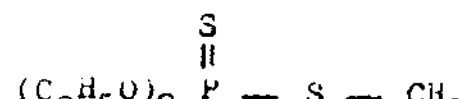


Ketothion

(Contd.)

43. Chlormephos

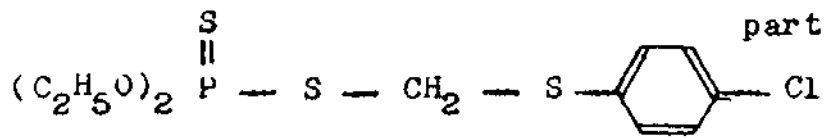
(Murphy chemical Co. 1968)



O,O-diethyl S - chloromethyl phosphorothiolothionate.

Liquid; non-systemic soil insecticide, particularly for control of larvae of coleoptera (wireworms); LD_{50}, 7 mg/kg.

44. Trithion(R), Garrathion(R), Carbophenothion, Akarithion(R) (Stanffer chemical Co. 1955).

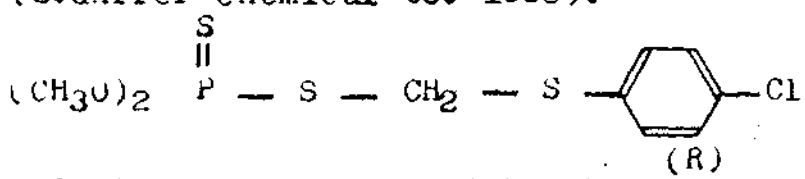


O,O-diethyl S - (p-chlorophenylthiomethyl) phosphorothiolothionate.

Liquid; insecticide and acaricide with long residual action; used for sucking plant pests, particularly mites; useful as a dip for cattle tick; LD_{50}, 30 mg/kg.

45. Methyl trithion(R), methyl carbophenothion

(Stanffer chemical Co. 1958).

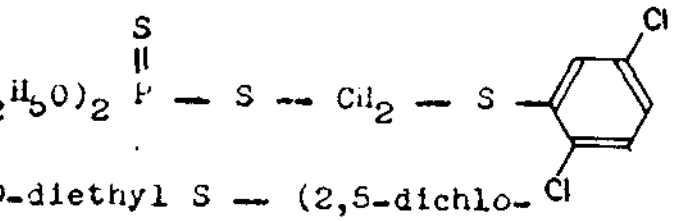


O,O-dimethyl homolog of trithion(R)

Liquid; for control of variety of insects and mites, particularly cotton boll weevil; LD_{50}, 150 mg/kg.

(Contd.)

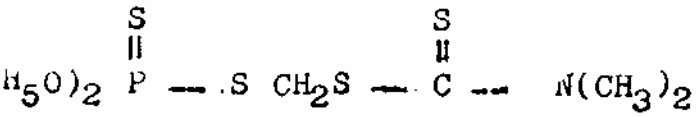
46. Phenkapton, Phencapton(R)
(Geigy AG. 1956)



O,O-diethyl S — (2,5-dichlorophenylthiomethyl) phosphorothiolothionate.

Liquid; non-systemic, selective acaricide with prolonged action, and effective against all development stages including eggs; LD_{50}, 200 mg/kg.

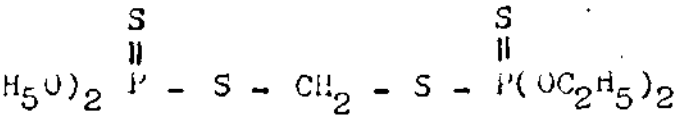
47. Azothion
(Farbwerke Hoechst. 1957)



O,O-diethyl S — (N,N-dimethylthiocarbamylthiomethyl phosphorothiolothionate.

Insecticide and acaricide, LD_{50}, 150 mg/kg.

48. Ethion, Nialate(R)
(Food Machinery and Chemical Corp. 1956)

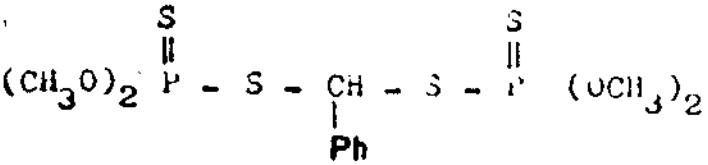


O,O,O',O' - tetraethyl S,S' - methylene. bis (phosphorothiolothionate)

Liquid; for control of aphids, scales, and mites; LD_{50}, 208 mg/kg.

(Contd.)

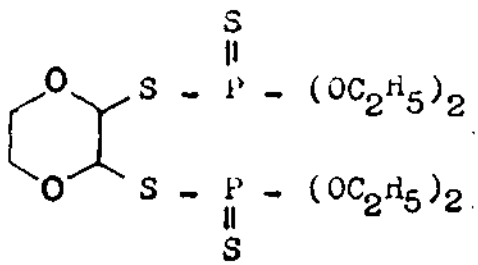
48. (Contd.)



Tetramethyl homolog is effective to bean leaf-lice and <u>Tetranychus telarius</u>.

The benzylidene derivative [SD-7438; tetramethyl S,S'-benzylidene bis (phosphorothilothionate)] is also an insecticide, LD_{50} - 280 mg/kg.

49. Dioxathion, Delnav(R), Navadel(R) (Hercules Incorporated. 1954).



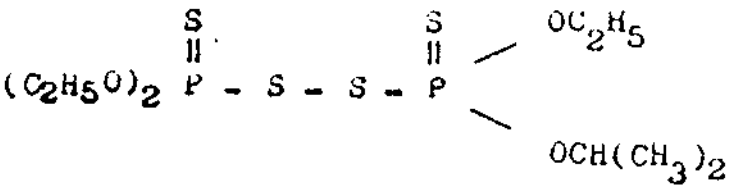
Liquid; non-systemic acaricide and insecticide with a long residual effect and is particularly useful to control mites on cotton and fruits and ticks, lice, and horn fly on cattle; the cis-isomer is more toxic to both insects and mammals than the transisomer; LD_{50}, 65-240 mg/kg (mixture of Cis- & trans-)

1,4 - dioxan - 2,3 - ylidene bis (O,O-diethyl phosphorothiolothionate) The methyl ester homolog has high insecticidal activity but low mammalian toxicity (LD_{50}, 300 mg/kg.).

(Contd.)

50. Phostex(R)

(Food Machinery and Chemical Corp. 1954).



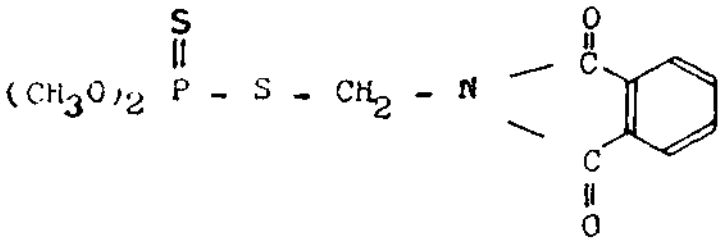
Diethoxyphosphinothionyl ethoxy-isopropoxy-phosphinothionyl disulphide.

The homologous bis(dialkyloxyphosphinothioyl) disulphides synergize the insecticidal activity of many organophosphorus insecticides.

weak contact insecticide, but a highly effective miticide with a good ovicidal activity; LD_{50}, 2,500 mg/kg.

51. Phosmet, Imidan(R), phthalophos, Prolate(R)

(Stanffer Chemical Co. 1966).



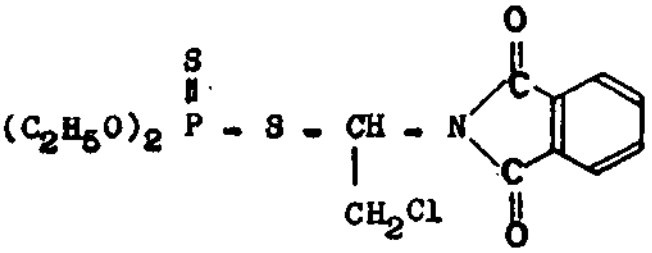
O,O-dimethyl S- (Phthalimidomethyl) phosphorothiolothionate.

White crystalline solid, m.p. 72°C; acaricide and broad spectrum insecticide for sucking and chewing insects; LD_{50}, 230 mg/kg.

(contd.)

52. Dialifos, Torak(R)

(Hercules Incorporated. 1965)

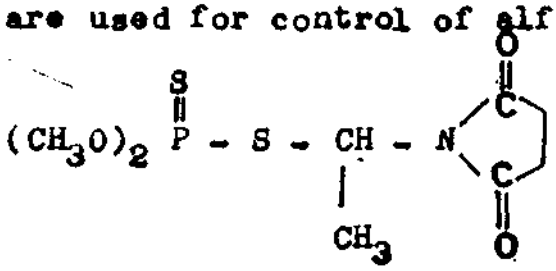


Crystalline solid m.p. 67-69°; insecticide and acaricide; LD_{50}, 5-97 mg/kg. (depending on species and sex).

O,O-diethyl S - (2-chloro-1-phtalimidoethyl) phosphorothiolothionate.

Methyl homolog as well as dialifor are used for control of alfalfa weevil.

Succinimide derivatives (Here. 13,462) are also effective insecticides.



Here - 13,462

53. (a) Azinphosmethyl, Guthion(R), Gusathion(R)

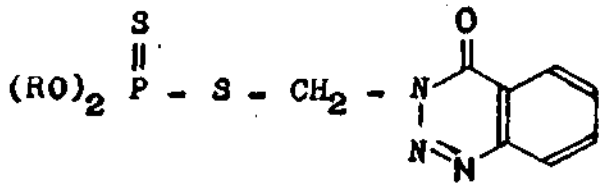
O,O-dimethyl S-4-oxobenzo-triazino-3 ylmethyl) phosphorothiolothionate.

Azinphosmethyl : white crystalline solid, m.p. 73-74°C, LD_{50}, 15 mg/kg.

53. Contd.

(b) Azinphosethyl, Ethyl Guthion, Gusathion A(R)

(Bayer AG. 1953)



R = CH_3 azinphosmethyl

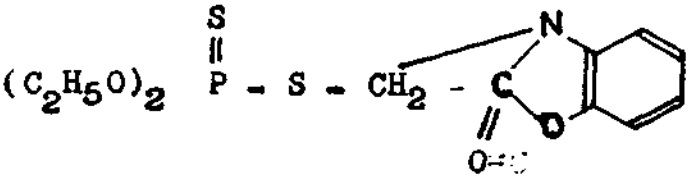
R = C_2H_5 azinphosethyl

Azinphosethyl; colourless needles, m.p. 53°C; LD_{50} 17.5 mg/kg.

Both are non-systemic insecticides and acaricides with long residual activity under neutral conditions.

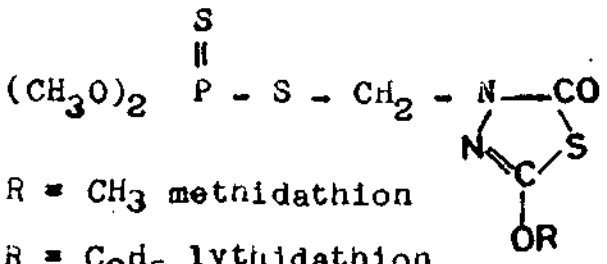
54. Zolone(R), Phosalone

(Rhone - Poulanc, France 1961).



O,O-diethyl S-(6-chloro-2-oxobenzoxazolin-3-ylmethyl) phosphorothiolothionate.

Solid; m.p. 39-40°C; non-systemic insecticide and acaricide with a wide spectrum; for controlling aphid; lepidopterous larvae (codling moth), and scales on fruit trees and cotton; LD_{50}, 25-48 mg/kg.



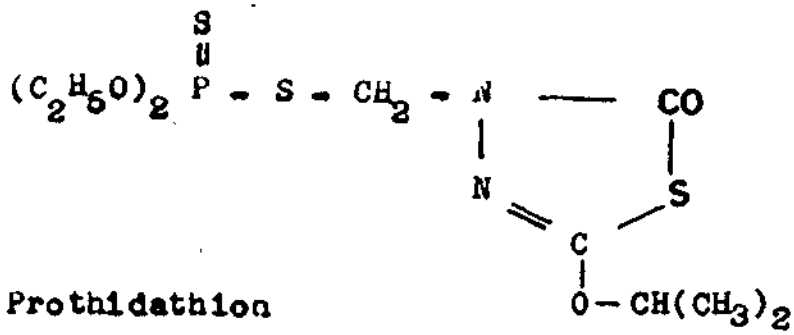
R = CH_3 methidathion

R = C_2H_5 lythidathion

Ethoxy homolog (lythidathion) (LD_{50}, 268-443 mg/kg) is useful specially against lepidopterous and dipterous larvae and otrhoptera.

(Contd.)

64. Contd.

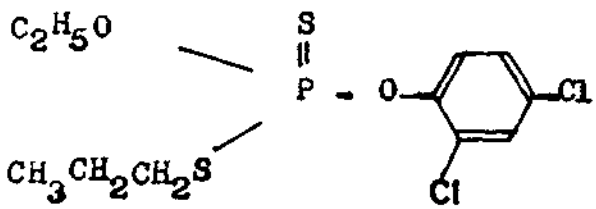


Prothidathion

Prothidathion, another homolog, is also useful as an insecticide.

65. (a) Tokuthion, Prothiofos (Bayer AG. 1975)

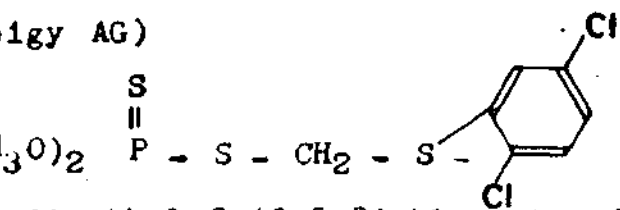
Experimental insecticide, effective to control mulbery mealybug, smaller tea tortrix, Caterpillers etc.; less toxic than the corresponding phosphorothionate.



O-ethyl S-Propyl O-(2,4-dichlorophenyl) phosphorothiolothionate.

(b) Methyl phenkapton (Geigy AG)

Acaricide and insecticide, LD_{50}, 375 mg/kg.

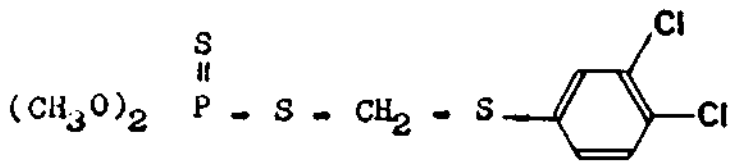


O,O-dimethyl S-(2,5-Dichlorophenylthiomethyl) phosphorothiolothionate.

(Contd.)

55.(b) Contd.

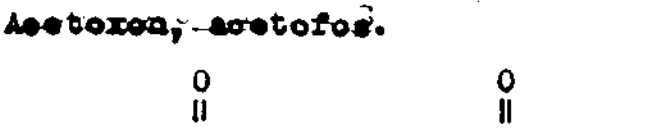
The 3,4-dichloro isomer of methyl phenkapton (G-30493) is an acaricide and may be useful as a fly larvicide in manure from chicks by oral administration.



G - 34093

56. PHOSPHOROTHIOLATES.

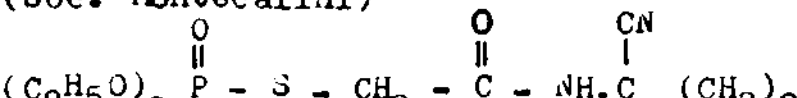
Acetoxon, acetofos.



O,O-diethyl S - ethoxy carbonylmethyl phosphorothiolate.

Liquid, contact insecticide; LD_{50}, 300-700 mg/kg Methylacetofos (dimethyl homolog) has also insecticidal property, but degraded very quickly during storage; LD_{50}, 100 mg/kg.

57. Cyanthoate, Tartan(R) (Soc. Montecalini)

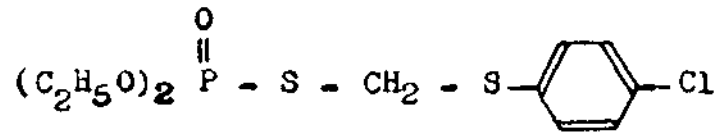


O,O-diethyl S - [N-(1-Cyano-1-methylethyl) Carbamoylmethyl] phosphorothiolate.

Liquid; insecticide and acaricide; LD_{50}, 3 to 4 mg/kg.

(Contd.)

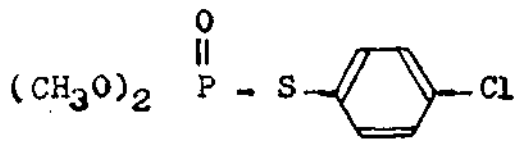
58. Danifos(R), PTMD
(Kumiai Chemical Co.)



O,O-diethyl S-(p-chlorophenylthiomethyl) phosphorothiolate

Liquid; acaricide; for control of spider mites, aphids and scale on citrus; LD_{50}, 165 mg/kg(M)

59. Fujithion(R), DMCP
(Kumiai Chemical Co. 1968)

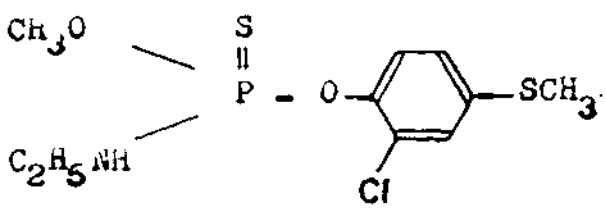


O,O-dimethyl S-(p-chlorophenyl) phosphorothiolates.

Liquid; insecticide, for control of leaf-hoppers and plant - hoppers which transmit rice virus diseases; LD_{50}, 100 mg/kg.

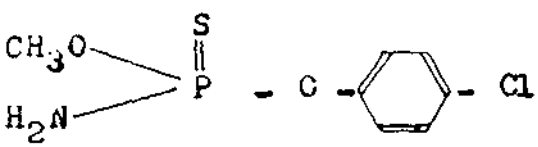
E. PHOSPHORAMIDATES

60. Amidothioate, Mitemate(R)
(Kippon Kayaku Co. 1965)



Yellow oil; miticide; long residual activity and can be mixed with Bordean mixture; kills both eggs and active mites; LD_{50}, 33 mg/kg (M)

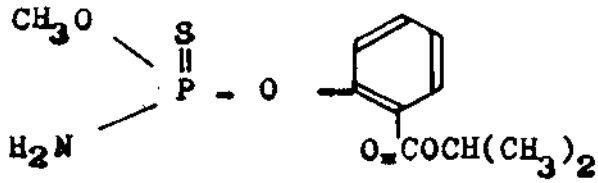
61. Dow ET - 15
(Dow Chem. Co.)



Insecticide; LD_{50}, 710 mg/kg.

(Contd.)

62. Optunal(R), isocarbophos



O-(Isopropoxycarbonyl) phenyl methyl phosphoramidothionate.

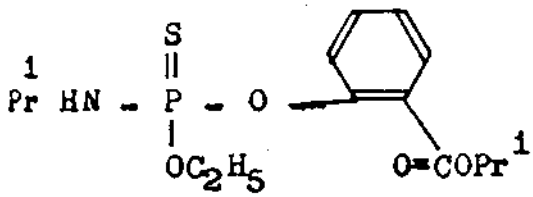
Effective for Colorado potato beetle and cotton insect; shows systemic activity against aphids and leaf rollers;

LD_{50}, 50-100 mg/kg.

<u>Other related alkyl phenyl phosphoramidates:</u>

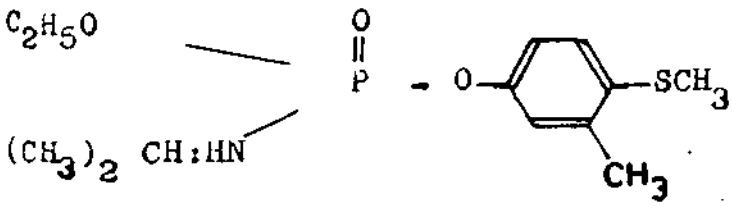
63. Isofenphos, Oftanol

(Bayer AG. 1974)



O-ethyl O-2-isopropoxycarbonyl-phenyl-isopropyl phosphoramidothioate.

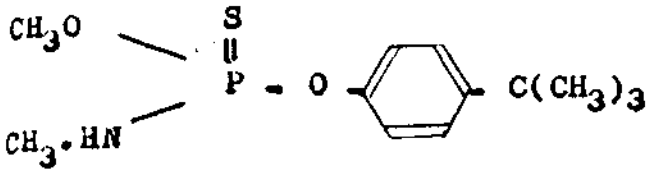
Colourless oil, broad-range non-systemic insecticide, effective against species of Lepidoptera, Colioptera, Diptera and to a variable extent Hemiptera, LD_{50}, 1800 mg/kg.



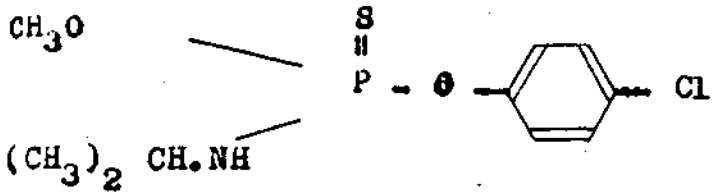
phenamiphos (nematocide)

(Contd.)

63. Contd.... .

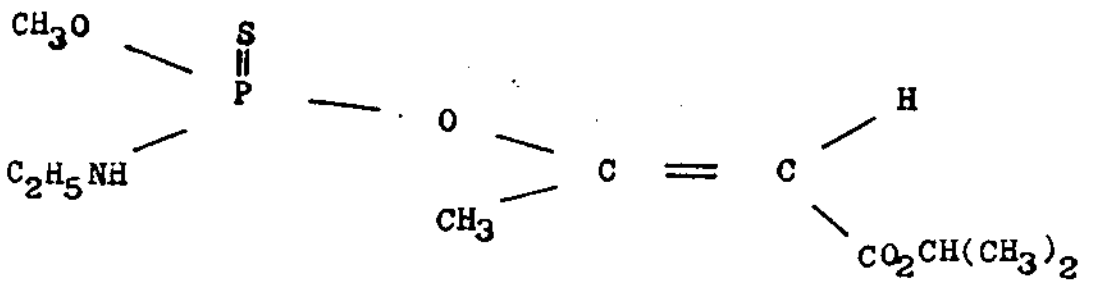


Narlene(R) (Animal systemic)



DMPA (herbicide)

64. Compound 577 (Sandoz, 1972)



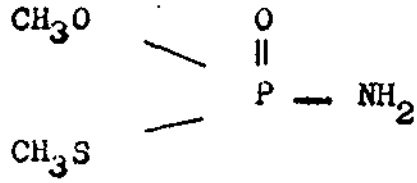
I-isopropoxycarbonyl-1-propen-2-yl methyl N-ethylphosphoramidothionate.

Experimental insecticide; trans-isomer is more toxic than cis-isomer; LD_{50}, 122 mg/kg (cis-isomer)

(Contd.)

64. Monitor(R), Tamaron(R), Methamidophos (Bayer AG. 1964; Chevron Research Co. 1969).

Solid, m.p. 44.5°C; acaricide and broad spectrum insecticide with contact and systemic action; LD_{50} 30 mg/kg.

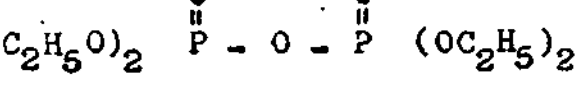


O,S - dimethyl phosphoramidothioate.

F. PYROPHOSPHATES.

65. TEPP, Tetron(R), (Bladan(R), Nifos T, Vapotone (Bayer AG. 1943)

Liquid, contact insecticide and acaricide; LD_{50} 1.2-2.0 mg/kg.
For homologs, insecticidal activity decreases : ethyl methyl propyl.



Tetraethyl pyrophosphate

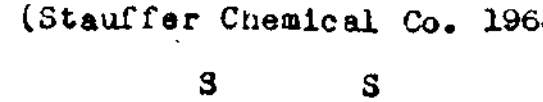
66. Sulfotep, Sulfotepp, Bladafum(R)

Liquid; insecticide and acaricide with high contact and fumigant activity; LD_{50}, 5 mg/kg.

O,O,O,O-tetra ethyl dithiopyrophosphate.

(Contd.)

67. Aspon(R), NPD(R),
Propyl thiopyrophosphate
(Stauffer Chemical Co. 1963).



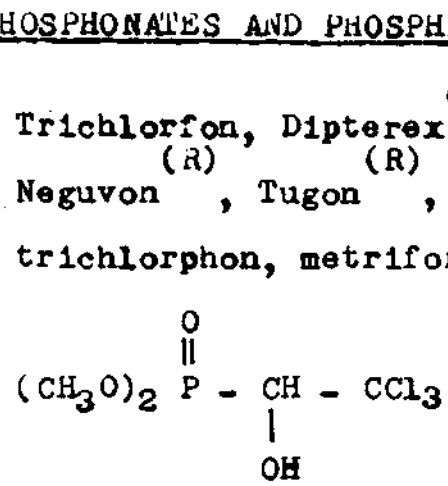
$$(C_3H_7O)_2\,P(=S)-O-P(=S)\,(OC_3H_7)_2$$

O,O,O - tetra-n-propyl dithiopyrophosphate.

Liquid; insecticide with contact and fumigant action; LD_{50}, 900-1700 mg/kg. Tetraisopropyl isomer has higher mammalian toxicity.

C. PHOSPHONATES AND PHOSPHINATES

68. Trichlorfon, Dipterex(R), Neguvon(R), Tugon(R), trichlorphon, metrifonate

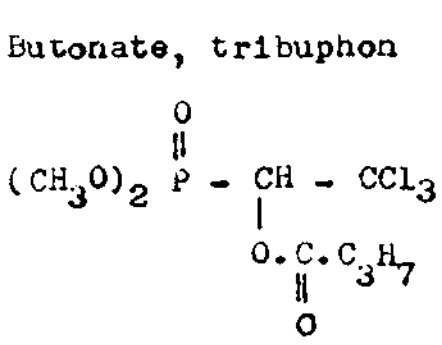


$$(CH_3O)_2\,P(=O)-CH(OH)-CCl_3$$

Dimethyl 1-hydroxy-2,2,2-trichloroethyl phosphonate.

Crystalline powder, mp 83-84°C high insecticidal activity, particularly against Diptera; useful for sucking and chewing insects, insects of public health and animal ectoparasites; LD_{50}, 630 mg/kg.

69. Butonate, tribuphon



$$(CH_3O)_2\,P(=O)-CH(O.C(=O).C_3H_7)-CCl_3$$

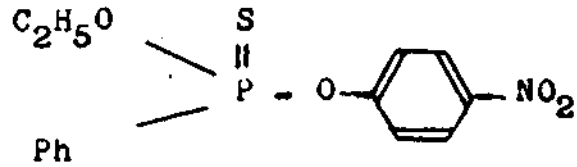
Dimethyl 1-n-butyryloxy-2,2,2-trichloroethylphosphonate.

Liquid; useful for cock-roaches, ants, flies and ectoparasites of domestic animals; LD_{50}, 1100 mg/kg.

(Contd.)

70. EPN

(Du Pont. 1949)

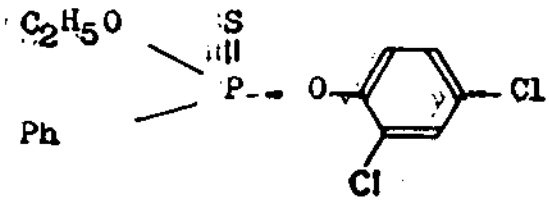


Ethyl p-nitrophenyl phenyl phosphonothionates.

Light yellow crystalline solid; mp. 36°C; insecticide and acaricide; potentiates the toxicity of malathion; shows delayed neurotoxicity; LD_{50}, 40 mg/kg (male rat) 12 mg/kg (female rat).

71. EPBP, S-Seven(R)

(Nissan Chemical Co. 1969).



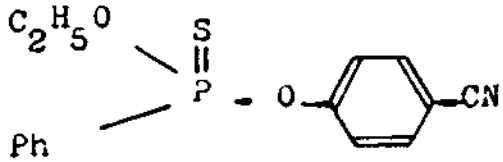
2,4-dichlorophenyl ethyl phenylphosphonothionate

Liquid; soil insecticide (onion maggot); LD_{50} 274 mg/kg (M).

Methyl ester homolog is more toxic than EPBP.

72. Cyanofenphos, CYP, Surecide(R)

(Sumitomo Chemical Co. 1966)

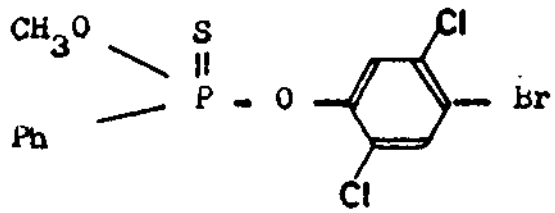


p-cyanophenyl ethyl phenylphosphonothionate.

White crystalline powder, mp. 83°C; insecticide — rice stem borer, cotton boll worm etc., LD_{50}, 80 mg/kg.

(Contd.)

73. Phosvel(R) Leptophos,
VCS - 506
(Velsicol Chemical Corp. 1965)

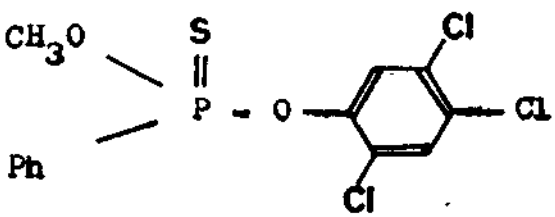


4-bromo-2,5-dichlorophenyl methyl phenylphosphonothionate.

White solid, mp - 70.2°C; insecticide; controlling lepidoptoran such as tobacco budworms, cotton leafworms, rice stem borers; has also fungicidal effect.
LD_{50}, 90 mg/kg.

It has no mutagenic or teratogenic properties.

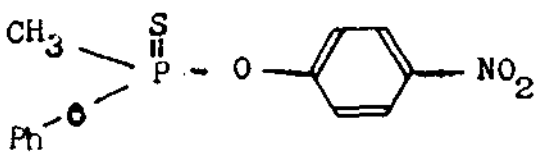
Closely related compound, methyl 2,4,5-trichlorophenyl phenylphosphonate (H 6034), is a fungicide.



H 6034

74. Colep(R)
(Monsanto Co. 1962)

Insecticide.

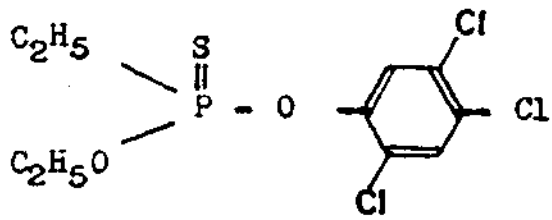


Phenyl p-nitrophenyl methylphosphonothionate.

(Contd.)

75. Trichloronate, Agritox(R), Agrisil(R), Phytosol(R) (Bayer AG. 1960)

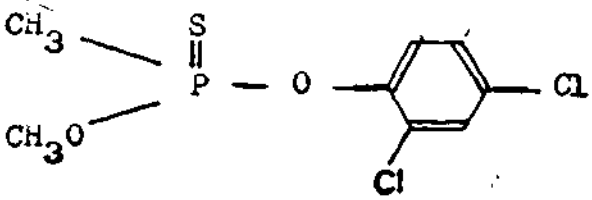
Liquid, non-systemic, persistent soil insecticide (root maggots and wireworms); LD_{50}, 50 mg/kg.



Ethyl 2,4,5 - trichlorophenyl ethylphosphonothionate.

76. Bay 30911

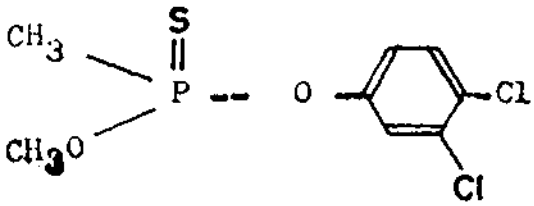
Systemic and contact insecticide with nematocidal activity; LD_{50}, 140 mg/kg.



Methyl 2,4-dicholorphenyl methylphosphonothionate.

7. Bay 80833

Soil insecticide.

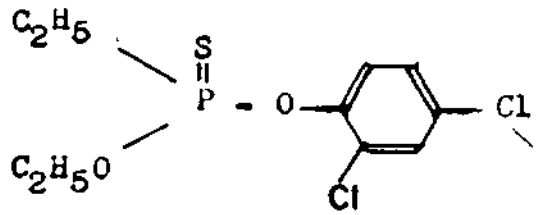


Methyl 3,4 - dichlorophenyl methylphosphonothionate.

(Contd.)

78. Stauffer N - 3054

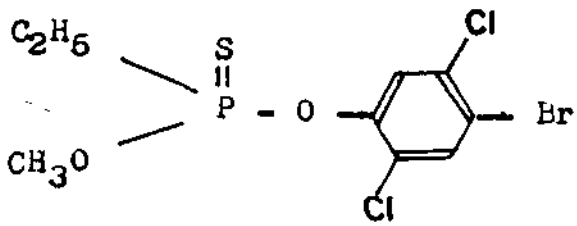
Insecticide ; LD_{50}, 75 mg/kg.



Ethyl 2,4-dichlorophenyl ethylphosphonothionate.

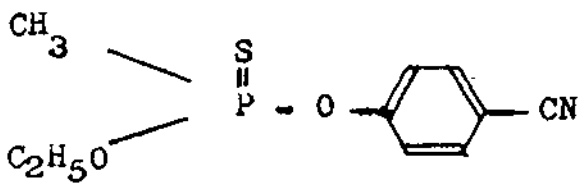
79. CELA K-41

Insecticide and acaricide, 7 times more active than trichloro analog; LD_{50}, 80 mg/kg.



80. Stauffer B - 10341

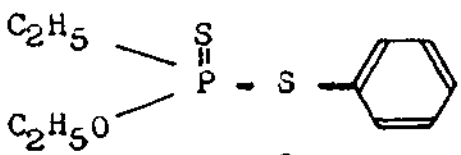
Insecticide, effective to resistant strain houseflies.



Ethyl 4-Cyanophenyl methylphosphonothionate.

81. Fonofox, Dyfonate(R)
(Stauffer Chemical Co. 1967)

Yellow liquid; non-systemic, persistant soil insecticide (corn root worms, wireworms, cutworms, maggots etc.)
LD_{50}, 36.5 mg/kg.



Fonofos

O-Ethyl S-phenyl ethylphosphonothiolothionate.

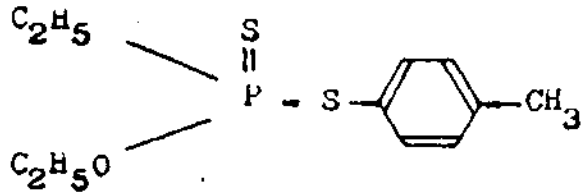
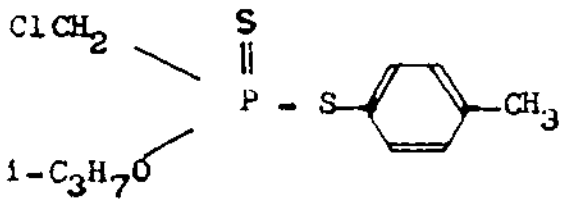
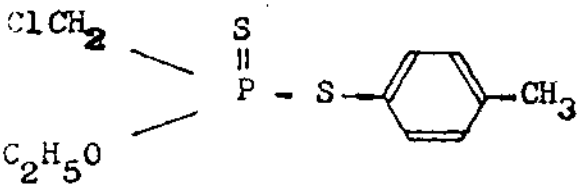
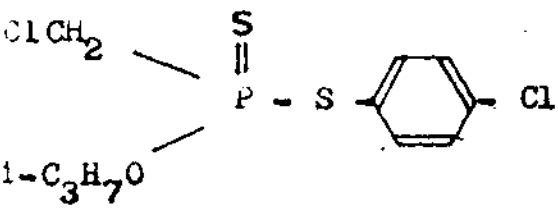
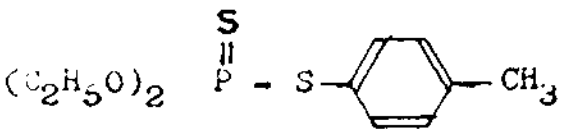
(Contd.)

Some other O-alkyl S-phenylphosphonothiolothionates:

		LD_{50} Rat mg/kg	LD_{50} Housefly mg/25 O+
$(C_2H_5)(C_2H_5O)P(=S)-S-Ph$		2.7	4.8
$(CH_3)(CH_3O)P(=S)-S-Ph$	N - 3727	141	7.5
$(C_2H_5)(CH_3O)P(=S)-S-Ph$	N - 3794	90	5.8
$(n-C_3H_7)(C_2H_5O)P(=S)-S-Ph$		55	8.8
$(C_2H_5)(C_2H_3O)P(=S)-S-C_6H_4-Cl$	N - 2596	6	5.0

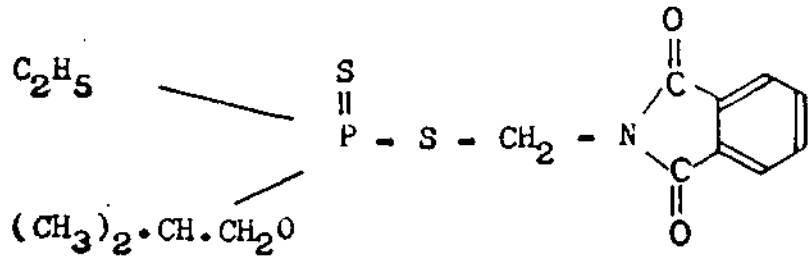
(Contd.)

81. Contd.....

	Bay 38156	123	4.3
	B - 10119	204	5.7
		336	5.4
	B - 10046	26	5.5
$(C_2H_5O)_2P(=S)-S-Ph$		233	56
		316	330

(Contd.)

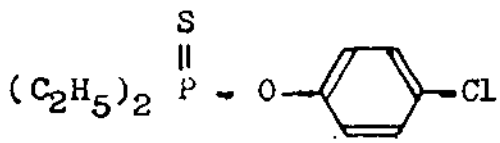
82. Stauffer N-4543



Solid, mp. 58-60°C, promising non-systemic insecticide and acaricide; LD_{50}, 75 mg/kg.

O - isobutyl S-(phthalimidomethyl) ethylphosphonothiolothionate.

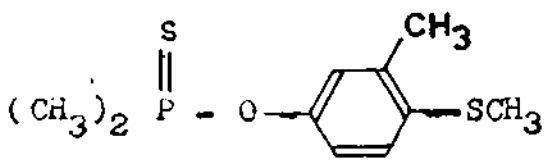
83. Agvitor
(Bayer AG. 1958)



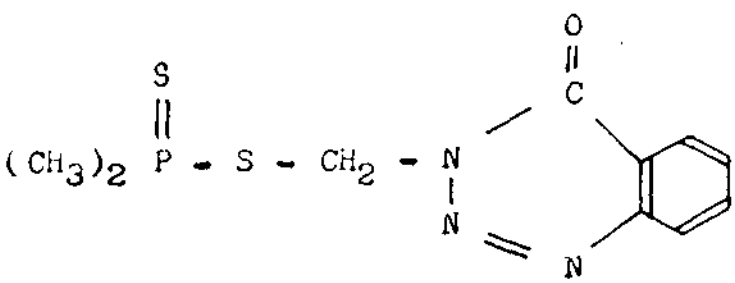
O-(2,4,5-trichlorophenyl) diethylphosphinothionate.

Liquid; soil insecticide (carrot and onion fly); LD_{50}, 100 mg/kg; (it is the phosphinate analog of ronnel).

<u>Other phosphinates:</u>



analog of fenthion; insecticide LD_{50}, 175 mg/kg



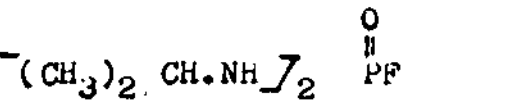
analog of azinphos-methyl. insecticide LD_{50}, 1000 mg/kg.

PLANT SYSTEMIC INSECTICIDES

Common or trade name, Chemical name and Chemical designations.	Physical and biological properties.
A: PHOSPHORAMIDES	
1. Schradan, OMPA, System(R) $[(CH_3)_2N]_2 P(=O) - O - P(=O) [N(CH_3)_2]_2$ N,N,N',N' - tetramethylphosphorodiamidie anhydride or Octamethyl pyrophosphoramide.	Liquid; effective against aphids and mites; biotransformed in vivo into toxic monomide oxide; weak contact action; LD_{50}, 13.5 to 35.5 mg/kg.
2. Dimefox, Hanane(R), Terra-System(R). (Fisons Pest Control Ltd. 1949). $[(CH_3)_2 N]_2 P(=O)F$ N,N,N',N' - tetramethylphosphorodiamidic fluoride	Liquid; extremely toxic; LD_{50} 1 to 5 mg/kg; formulated as gelaline ampoules for safe application in soil.

(Contd.)

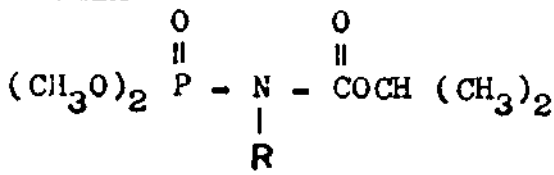
3. Mipafox, Isopestox(R), Pestox 15.
(Fisons Pest Control. 1950).



N, N - di-isopropylphosphorodiamidic fluoride.

Solid, mp. 61-62°C, not used now because of the delayed neurotoxicity.

4. Avenin



R = H avenin

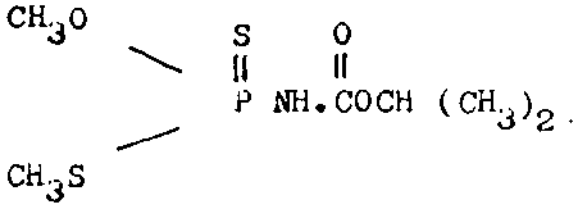
R = CH_3 demuphos

Dimethyl N-(isopropoxycarbonyl) phosphoramidiate.

Undistillable oil; insecticide, selective to sugar beet weevil and sensitive to bean aphids; LD_{50}, 5000 mg/kg.

Demuphos is more effective and persistent than avenin; can control sugar beet weevils for 20 - 25 days.

Another phosphoryl urethan which has insecticidal activity is:

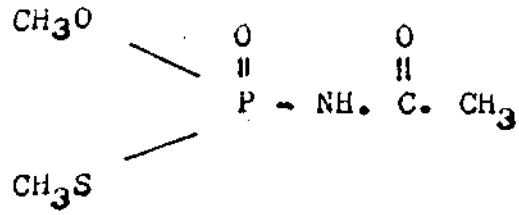


(Contd.)

5. Acephate, Orthene(R), Ortran(R)

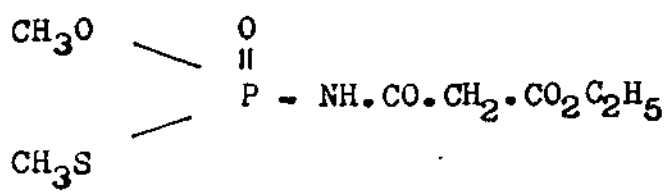
(Chevron Chemical Co. 1971).

It is the N-acetyl derivative of Monitor(R); solid, mp. 91-92°C; systemic insecticide; moderate persistence; used for sucking and chewing pests.
LD_{50}, 945 mg/kg (R), 361 mg/kg (M).



O,S - dimethyl N-acetylphosphoramidothiolate.

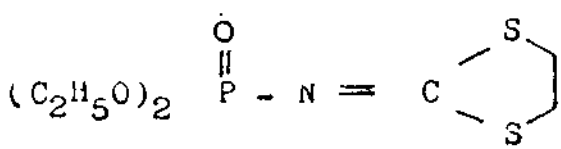
The N-malonyl ethyl ester derivative of Monitor(R) has a very good cuticular penetrating property:



6. Phosfolan, Cyolane(R)

(American Cyanamid Co. 1963).

Liquid; insecticide - cotton leafworms, sucking insects and mites; use also for soil insects, relatively long persistence in soil.
LD_{50}, 9 mg/kg.

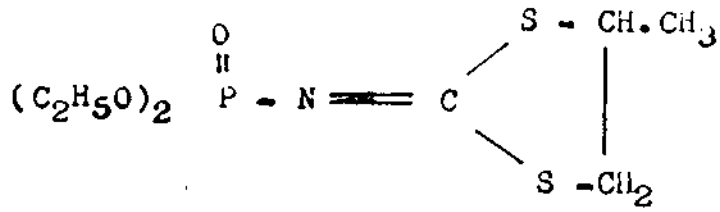


2-(diethoxyphosphinylimino)-1-3-dithiolene.

(Contd.)

7. Mephosfolan, Cytrolane(R)

(American Cyanamid Co.)

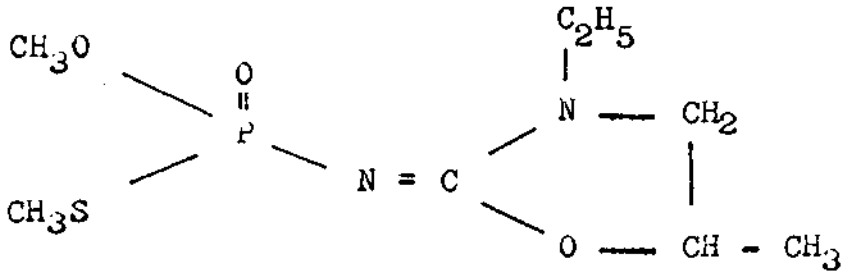


0,0 - diethyl (4-methyl - 1,3-dithiolan-2 ylidene) phosphoramidate.

Liquid; systemic insecticide and miticide; soil use against rice pests hopaphid etc.

LD_{50}, 3 to 9 mg/kg.

8. Stauffer R - 16,661



2-(methoxymethylthiophosphinylimino)-3-ethyl-5-methyl-1,3-oxazoline.

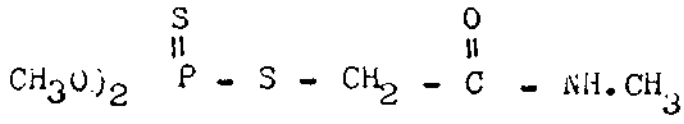
Systemic insecticide for sucking and chewing pests of cotton;

LD_{50}, 0.1 to 0.2 mg/kg (M)

B: CARBOXYAMIDES AND RELATED COMPOUNDS:

9. Dimethoate, Roger(R)

Cygon(R),



0,0 - dimethyl S - (N-methylcarbomoylmethyl) phosphorothiolothionate.

Solid, mp. 51-52°C; systemic and contact broad-range insecticide and acaricide, LD_{50}, 600 mg/kg (pure compound) 150 - 300 mg/kg (Technical product.).

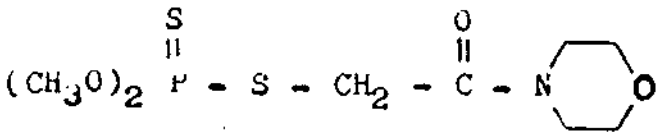
(Contd.)

10. **Folimat**(R) Omethoate (Bayer AG. 1965) $(CH_3O)_2\ \overset{O}{\overset{\|}{P}} - S - CH_2 - \overset{O}{\overset{\|}{C}} - NH.CH_3$ O,O - dimethyl S-(N-methylcarbamoylmethyl) phosphorothiolate.	Undistillable oil; systemic insecticide and acaricide with a broad-range of action hopaphid and wheat bulb fly; LD_{50}, 50 mg/kg.
11. Ethoate-methyl, Fitios B/77 (Bombrini Parodi-Delfino, Italy). $(CH_3O)_2\ \overset{S}{\overset{\|}{P}} - S - CH_2 - \overset{O}{\overset{\|}{C}} - NH.C_2H_5$ O, O - dimethyl S - (N-ethylcarbamoylmethyl) phosphorothiolothionate.	Solid; m.p. 67-68°C; insecticide and acaricide with contact action, particularly effective against fruit fly; LD_{50}, 340 mg/kg.
12. Prothoate, Fostion(R), Fac 20(R) (Soc. Montecatini. 1956). $(C_2H_5O)_2\ \overset{S}{\overset{\|}{P}} - S - CH_2 - \overset{O}{\overset{\|}{C}} - NH.CH(CH_3)_2$ O, O - diethyl S-(N-isopropylcarbamoylmethyl) phosphorothiolothionate. The O - methyl homolog (m.p. 77-78°C) has also insecticidal property; LD_{50}, 250 mg/kg.	Crystalline solid, m.p. 28.5°C; acaricide and insecticide with systemic action, used mainly against phytophagous mites and some sap-sucking insects; olive fly, cherry maggot;

(Contd.)

13. Morphothion, Ekatin F(R), Morphotox(R)
(Sandoz Ltd., 1956)

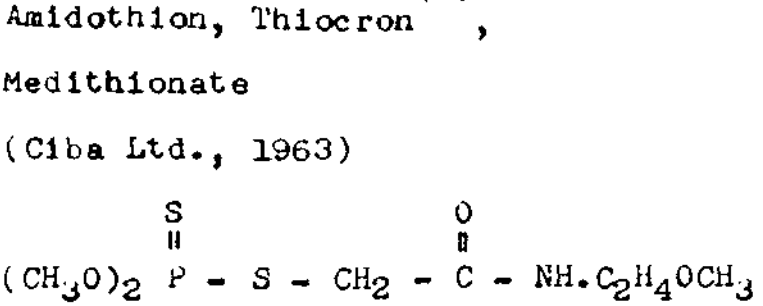
Crystalline solid, m.p. 63-64°C; a systemic and contact insecticide; LD_{50}, 190 mg/kg.



O, O-dimethyl S-(morpholinocarbonylmethyl) phosphorothiolothionate.

14. Amidothion, Thiocron(R), Medithionate
(Ciba Ltd., 1963)

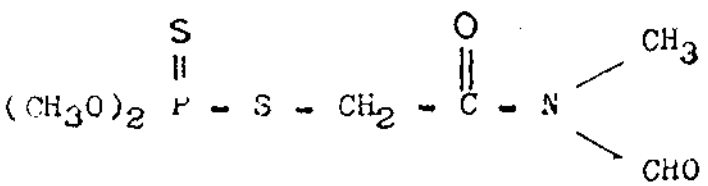
Solid, m.p. 46°C; insecticide, sucking pests on cherries and vegetables; LD_{50}, 600-660 mg/kg.



O, O-dimethyl S-(N-methoxyethylcarbamoylmethyl phosphorothiolothionate.

15. Formothion, Anthio(R), Aflix(R)
(Sandoz Ltd., 1963)

Liquid; contact and systemic acaricide and insecticide, effective against both sucking and chewing pests; LD_{50}, 375 - 535 mg/kg.



O,O-dimethyl S-(N-formyl-N-methylcarbamoylmethyl) phosphorothiolothionate.

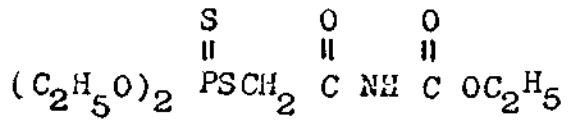
16. Mecarbam, Pestan(R), Murfotox(R), Afos(R)

(Murphy Chemical Ltd., 1961)

O, O-diethyl S-(N-ethoxycarbonyl-N-methyl-carbamoylmethyl) phosphorothiolothionate.

Liquid; contact insecticide and acaricide with slight systemic action; persists in soil for several weeks; useful for control of sucking and chewing pests and root maggots of vegetables; LD_{50}, 36 mg/kg.

17. Bay 19596

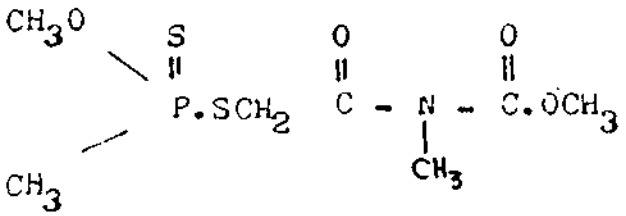


N-desmethyl homolog of Mecarbam

Insecticide, effective against the oriental fruit fly and the melonfly.

18. Mecarphos, Mecarphon

(Murphy Chemical Ltd.,)



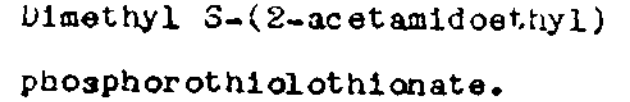
[S - (N - methoxycarbonyl - N - methylcarbamoylmethyl)] methyl methylphosphorothiolothionate.

Systemic insecticide; LD_{50}, 57 mg/kg.

(Contd.)

19. DAEP, Amiphos(R)

(Nippon Soda Co. 1966)

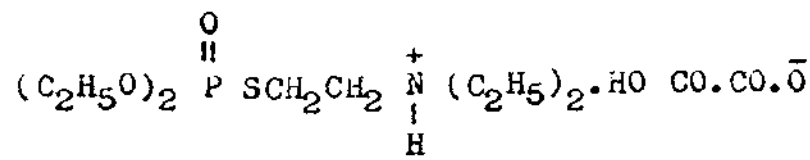


Dimethyl S-(2-acetamidoethyl) phosphorothiolothionate.

Solid, mp. 22-23°C; systemic acaricide and insecticide, useful to control sucking pests on fruits, vegetables, and ornamentals; LD_{50}, 400 mg/kg.

20. Amiton, Tetram(R), Citram(R).

(Imperial Chemical Industries)

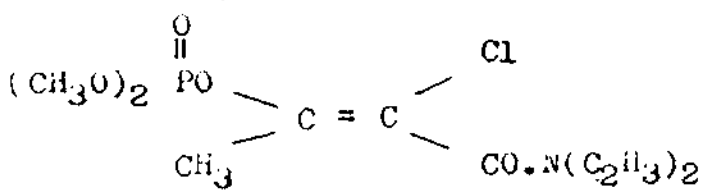


Diethyl S - (2-diethylaminoethyl) phosphorothiolate hydrogen oxalate

Solid, mp. 100-101°C (Free base liquid); long acting systemic insecticide and acaricide; LD_{50}, 0.5 - 7 mg/kg (free base, for various animals).

21. Phosphamidon, Dimecron(R), Dicron(R)

(Ciba Ltd.)

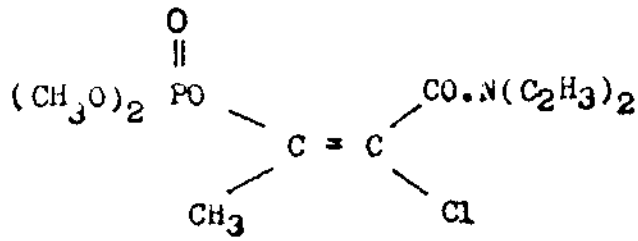


Cis(β-) -isomer

Liquid; systemic insecticide and acaricide with a little contact action; useful against sap-feeding and leaf-eating insects, particularly aphids, bugs, caterpillars (not Noctuidae), thrips rice stem borers etc. LD_{50}, 15-28 mg/kg.

21. Contd.....

0,0-dimethyl 0-(1-methyl-2-chloro-2-diethylcarbamoyl vinyl) phosphate

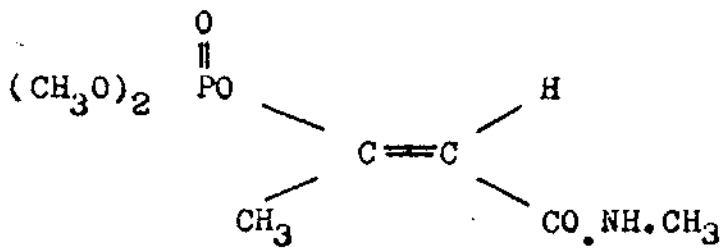


The cis - isomer is more biologically active than trans - isomer.

tans (α-) - isomer

22. Monocrotophos, Azodrin(R), Nuvacron(R)

(Ciba AG. 1965)



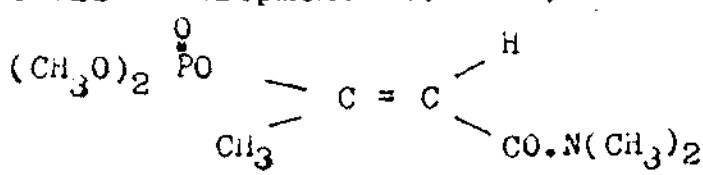
3- (dimethoxyphosphinyloxy) - N - methyl-cis-crotonamide.

Solid, mp. 53-55; a fast-acting insecticide with systemic, stomach and contact action, used against a wide range of pests; LD_{50}, 21 mg/kg.

23. Dicrotophos, Bidrin(R) Ektafos(R), Carbicron(R).

(Ciba AG. 1963)

(Shell Development Co. 1965)



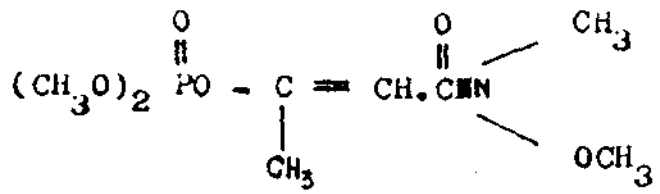
3-(dimethoxyphosphinyloxy)-N,N-dimethyl-cis-crotonamide.

Liquid; moderately persistent systemic insecticide with stomach and contact poison and useful for controlling sucking, chewing, and mining insects and certain mites; LD_{50}, 22 mg/kg.

(Contd.)

24. C - 2307 — Insecticide; LD_{50}, 2 mg/kg.

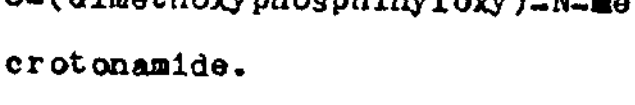
(Ciba AG)



3-(dimethoxyphosphinyloxy)-N-Methoxy-N-methyl-crotonamide.

25. Phosdrin(R), Mevinphos — Liquid; a contact and systemic insecticide and acaricide of short persistence, effective against sucking and chewing insects and mites; LD_{50}, 4-7 mg/kg.

(Shell Development Co. 1953)

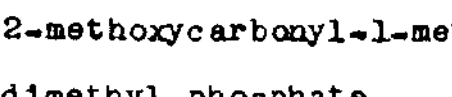


2-methoxycarbonyl-1-methylvinyl dimethyl phosphate.

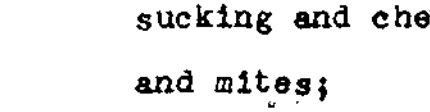
C: SULPHIDES AND RELATED COMPOUNDS.

26. Demeton, Systox(R), mercaptophos — Liquid; technical product contains 70% thiono-isomer and 30% thiolo-isomer; LD_{50} 30 mg/kg (demeton-O) and LD_{50}, 1.5 mg/kg (demeton-S); it is a systemic and acaricide with some

(Bayer AG. 1951)



demeton - S



demeton - O

(Contd.)

26. Contd.....

or

Isosystox(R)

(Thiolo-isomer)

O,O-diethyl 2-ethylthioethyl phosphorothioate.

fumigant action, effective especially against sap-sucking insects and mites.

27. Demeton-methyl, Metasystox(R), (Bayer AG. 1954)

$(CH_3O)_2\overset{O}{\overset{\|}{P}}S\ CH_2\ CH_2SC_2H_5$ $(CH_3O)_2\overset{S}{\overset{\|}{P}}OCH_2CH_2SC_2H_5$

or

Metasystox 55

or

Isometasystox

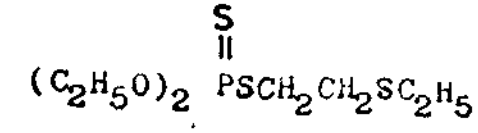
O,O-dimethyl 2-ethylthoethyl phosphorothioate.

Liquid, technical product contains 70:30 mixture of thiono-isomer and thiolo-isomer; LD_{50}, 40 mg/kg (thiolo-isomer); it is a systemic and contact insecticide and acaricide, rapid in action, moderate persistence, effective against sap-sucking pests, sawflies, red spider mites etc.

(Contd.)

28. Demephion, methyldemeton-methyl, Tinox(R), Cymetox(R) (Farbenfabriken Wolfen, East Germany). $(CH_3O)_2\overset{O}{\overset{\|}{P}}SCH_2CH_2SCH_3$ demephion - S (thiolo-isomer) $(CH_3O)_2\overset{S}{\overset{\|}{P}}OCH_2CH_2SCH_3$ demephion - O (thiono-isomer) O,O-dimethyl 2-methylthioethyl phosphorothioate.	Liquid; technical product is a mixture of thiono-isomer (30 to 40%) and thiolo-isomer (60 to 70%); LD_{50}, 750 mg/kg (thiono-isomer), LD_{50}, 40 mg/kg (thiolo-isomer); systemic insecticide and acaricide; sap-feeding insects.
29. Thiometon, Ekatin(R), Dithoometasystox(R) (Sandoz Ltd.). $(CH_3O)_2\overset{S}{\overset{\|}{P}}SCH_2CH_2SC_2H_5$ O,O-dimethyl S-(2-ethylthioethyl) phosphorothiolothionate.	Liquid; a systemic insecticide and acaricide with contact action; effective against aphids, sawflies, thripe, and mites; LD_{50}, 70-120 mg/kg.

30. Disulfoton, thiodemeton, Disyston(R), Dithiosystox (Bayer AG. 1956)

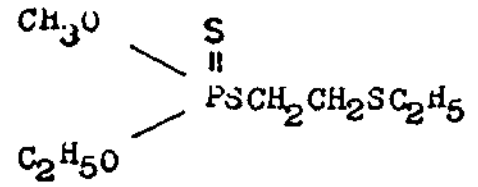


$(C_2H_5O)_2\ PSCH_2CH_2SC_2H_5$

O,O-diethyl S-(2-ethylthioethyl) phosphorothiolothionate.

Liquid; a systemic insecticide and acaricide, used mainly for soil pests; seed dressing or granules to protect seedlings; effective against aphids on vegetables and fruits and carrot fly, leafhoppers on rice, vegetables, cotton and for some other pests.

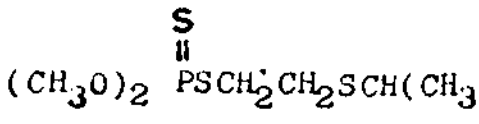
31. Tetrathion (in Czechoslovakia)



O - methyl O-ethyl S-(2-ethylthioethyl) phosphorothiolothionate.

Liquid; a systemic insecticide and acaricide, used mainly for soil pests; chemical and insecticidal properties are similar to thiometon and disulfoton; LD_{50}, 1.2 - 22.3 mg/kg (various animals).

32. Isothioate, Hosdon(R) (Nihon Noyaku Co. 1972)



$(CH_3O)_2\ PSCH_2CH_2SCH(CH_3)_2$

O,O-dimethyl S-(2-isopropylthioethyl) phosphorothiolothionate.

Liquid; a systemic insecticide and acaricide, effective against sucking plant pests by soil treatment or foliage spray; LD_{50}, 200 mg/kg.

(Contd.)

33. Phorate, Thimet(R), timet (American Cyanamid Co. 1954).

$(C_2H_5O)_2\ \overset{S}{\overset{\|}{P}}SCH_2SC_2H_5$

O,O-diethyl S-(ethylthiomethyl) phosphorothiolothionate.

Liquid; a persistent systemic insecticide, used for the protection seedlings from sap-feeding and soil insects; it has also some contact and fumigant action; LD_{50}, 2-4 mg/kg.

34. Am. Cy. 12008 (American Cyanamid Co. 1954)

$(C_2H_5O)_2\ \overset{S}{\overset{\|}{P}}SCH_2S\ CH(CH_3)_2$

O, O - diethyl S-(isopropylthiomethyl) phosphorothiolothionate.

Liquid; systemic insecticide with some contact activity, LD_{50}, 14 to 16 mg/kg (M).

35. Aphidan, IPSP, PSP - 204 (Hokko Chemical Co. 1964)

$[(CH_3)_2\ CHO]_2\ \overset{S}{\overset{\|}{P}}SCH_2\ \overset{O}{\overset{\uparrow}{S}}C_2H_5$

O,O-di-isopropyl S-(ethylsulfinylmethyl) phosphorothiolothionate.

Liquid; a systemic insecticide, applied in soils to protect the plant from the attack of aphid; potatoes can be protected from the aphid vector of virus diseases by treating the seed potatoes; LD_{50}, 84.5 mg/kg.

36. Oxydisulfoton, Disystons(R), Disyston-sulfoxide(R) (Bayer AG. 1965)

$(C_2H_5O)_2\ \overset{S}{\overset{\|}{P}}SCH_2CH_2\ \overset{O}{\overset{\uparrow}{S}}C_2H_5$

O,O-diethyl S-(2-ethylsulfinylethyl) phosphorothiolothionate.

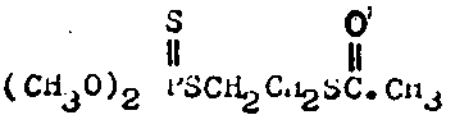
Liquid; a persistent, systemic insecticide and acaricide particularly suitable for seed treatment against virus vectors (sap-sucking insect); LD_{50}, 3.5 mg/kg.

(Contd.)

37. Oxydemeton-methyl, Metasystox - R(R) Isometasystox-sulfoxide, demeton-S-methyl-sulfoxide. (Bayer AG. 1960). $(CH_3O)_2\ P(=O)SCH_2CH_2\ S(\uparrow O)C_2H_5$ O,O-dimethyl S-(2-ethylsulfinylethyl) phosphorothiolate.	Liquid; a systemic and contact insecticide, used against sap-sucking insects and mites, with a fast kill and moderate persistence; LD_{50}, 70 mg/kg.
38. Metasystox - S(R), Estox(R) (Bayer AG. 1955) $(CH_3O)_2\ P(=O)SCH_2CH_2\ S(\uparrow O)C_2H_5$ O, O-dimethyl S-(2-ethylsulfinylisopropyl) phosphorothiolate.	Liquid; a systemic and contact insecticide, like oxydemetonmethyl, LD_{50}, 105 mg/kg.
39. Dioxydemeton - S - methul, Demeton - S - methyl - sulfone, Metaisosystox - sulfon. (Bayer AG. 1965) $(CH_3O)_2\ P(=O)SCH_2\ CH_2\ S(\uparrow O)(\downarrow O)C_2H_5$ O, O-dimethyl S-(2-ethylsulfonylethyl) phosphorothiolate.	Solid, mp. 60°C; a systemic insecticide; LD_{50}, 37.5 mg/kg.

(Contd.)

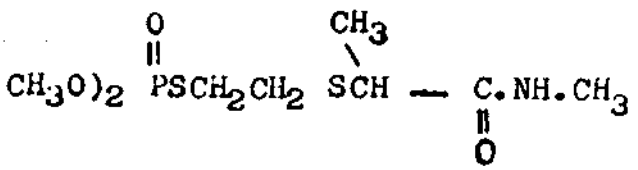
40. Intration (in Czechoslovakia, 1967)



0,0-dimethyl S-(2-acetylthioethyl) phosphorothiolothionate.

Systemic insecticide for aphid control on hop.

41. Vamidothion, Kilval(R) (Rhone - Poulenc, France. 1962).

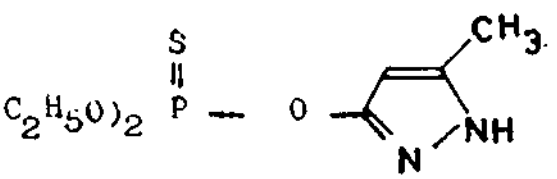


0,0-dimethyl S- [2-(1-methylcarbamoyl-ethylthio) ethyl] phosphorothiolate.

Solid, mp. 46 to 48°C; a systemic insecticide and acaricide of high persistence of particular value against wooly aphid; the sulfoxide has similar biological activity but greater persistence; LD_{50}, 64 to 100 mg/kg.

D: PHOSPHORUS ESTER CONTAINING HETERO AROMATIC RING:

42. Pyrazothion(R) (Geigy Chem. Corp. 1952)



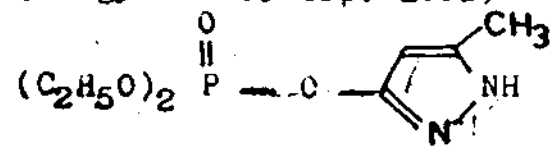
0,0-diethyl 3-methylpyrazol-5-yl phosphorothionate.

Liquid; systemic insecticide, effective against aphids and spider mites; LD_{50}, 36 mg/kg.

(Contd.)

43. Pyrazoxon(R)

(Geigy Chem. Corp. 1952)

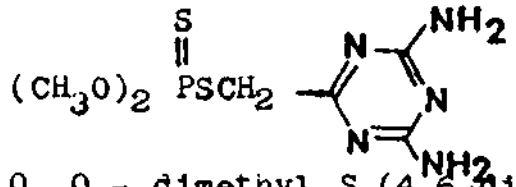


0, 0 - diethyl 3-methylpyrazol-5-yl phosphate.

Liquid; systemic insecticide; LD_{50}, 4 mg/kg.

44. Menazon, azidithion, Saphizon(R), Sayfos(R)

(Plant Protection Ltd. 1959)

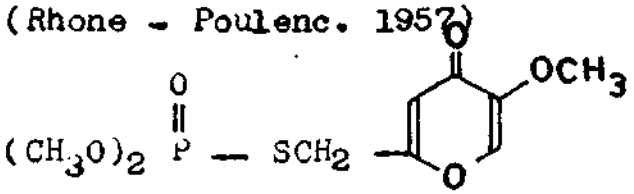


0, 0 - dimethyl S-(4,6-diamino-1,3,5-triazin-2-ylmethyl phosphorothiolothionate.

Crystalline solid, mp. 166°C; ystemic aphicide, used by foliar spray, soil treatment, seed dressing, and root dip; LD_{50}, 900 to 1,950 mg/kg.

45. Endothion, Exothion(R), Endocide(R), Phosphopyron(R)

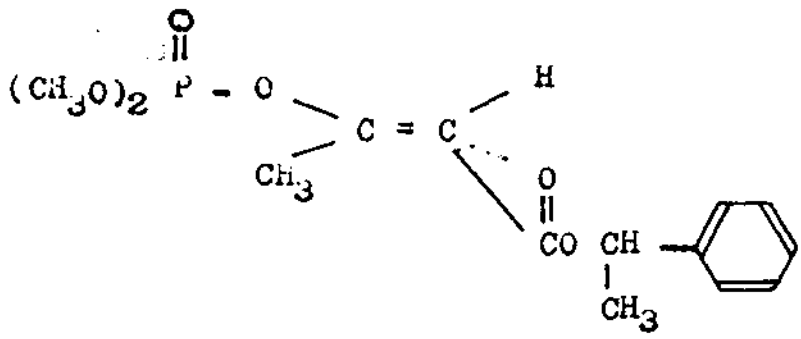
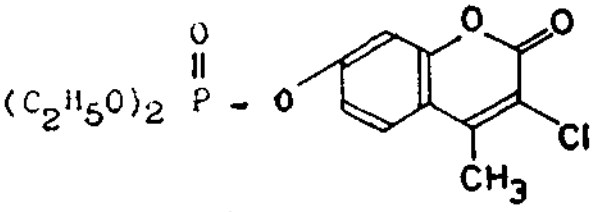
(Rhone - Poulenc. 1957)



0, 0 - dimethyl S - (5-methoxy-4-0x0-4H-pyran-2-ylmethyl) phosphorothiolate.

Solid, mp. 90 to 91°C; systemic insecticide and acaricide; LD_{50}, 30 - 50 mg/kg.

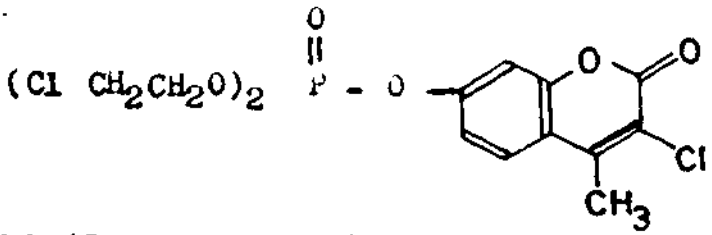
ANIMAL SYSTEMIC INSECTICIDE AND VETERINARY PESTICIDES.

Common or trade name, Chemical name and Chemical designations.	Physical and biological properties.
A: PHOSPHATES:	
1. Crotoxyphos, Ciordrin(R) (Shell Development Co. 1962).  Dimethyl cis -1-methyl-2-(1-phenylethoxy-carbonyl) vinyl phosphate.	Liquid; insecticide with no systemic activity, effective against ectoparasites on livestock by spray (for the control of flies, lice, mites, and ticks on cattle and pigs by spray); LD_{50}, 125 mg/kg.
2. Coroxon (Cooper Technical Bureau. 1961)  Diethyl 3-chloro-4-methylcoumarin-7-yl phosphate.	Solid, mp. 72°C; animal systemic insecticide; LD_{50}, 12 mg/kg.

(Contd.)

3. Haloxon

(Cooper Tech. Bureau. 1962)

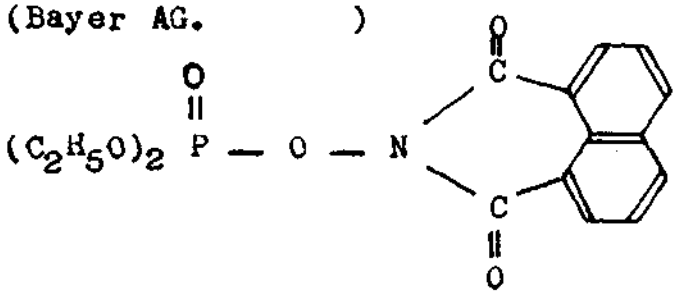


Bis(2-chloroethyl) 3-chloro-4-methyl-coumarin-7-yl phosphate.

Solid, mp. 92°C; has high anthelmintic properties, but has no useful insecticidal properties; LD_{50}, 900 mg/kg.

4. Maretin(R), Rametin(R),

(Bayer AG.)

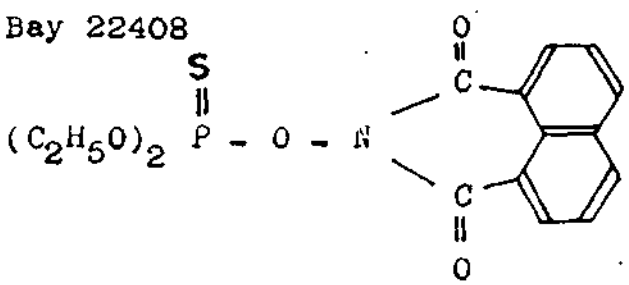


N-(diethoxyphosphinyloxy)-naphthalimide

Solid, mp. 196°C; has anthelmintic and insecticidal properties; LD_{50}, 75 mg/kg.

B: PHOSPHOROTHIONATES.

5. Bay 22408



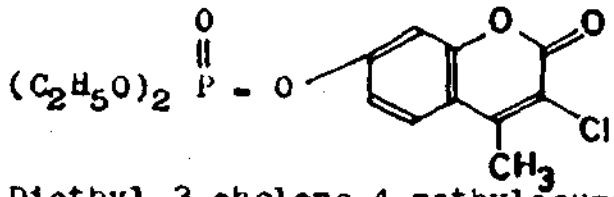
N-(diethoxyphosphinothioyloxy)-naphthalimide.

Solid; insecticide, effective against Mediterranean fruit flies; LD_{50}, 500 mg/kg.

(Contd.)

6. Coumaphos, Co-Ral(R), Asuntol(R), Muscatox(R)

(Montecatini. 1956)

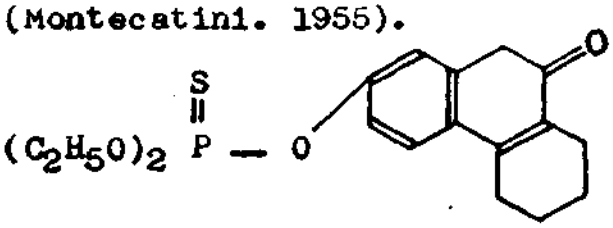


Diethyl 3-choloro-4-methylcoumairn-7-yl phosphorothionate.

Solid, mp. 95°C; insecticide, useful to control ectoparasites on cattle, goats, etc. by feed or spray; also effective against flies, mosquito larvae; active against gastrointestinal nematodes and has synergistic activity with the anthelmintic agent phenothiazine; LD_{50}, 90 to 110 mg/kg.

7. Coumithoate, Dition(R)

(Montecatini. 1955).

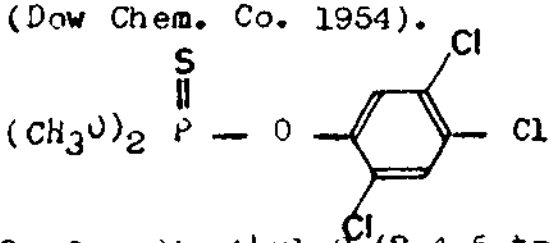


O,O-diethyl O-(3,4-tetramethylene-7-coumarinyl) phosphorothionate.

Solid, mp. 88-89°C; insecticide and meticide, useful for livestock pest control and for public health; LD_{50}, 150 mg/kg.

8. Ronnel, Trolene(R), Fenchlorphos, Nankor(R), Korlan(R)

(Dow Chem. Co. 1954).

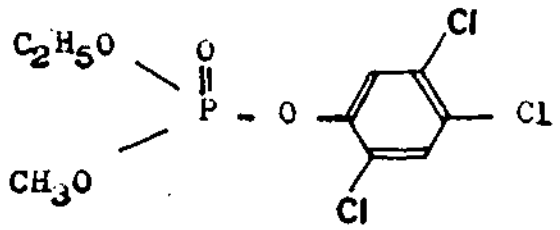


O, O - dimethyl O-(2,4,5-trichlorophenyl) phosphorothionate.

Solid, mp. 40-42°C; animal systemic insecticide, effective to control cattle grub, lice, hornfly, screw-worm, and ticks on livestock by oral administration: LD_{50}, 1250-1750 mg/kg

(Contd.)

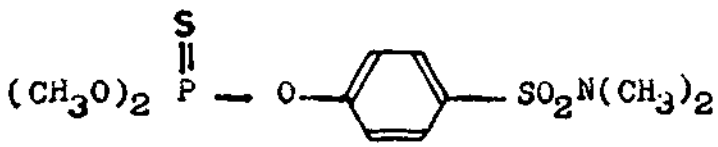
9. Trichlormetaphos - (USSR)



Liquid; insecticide, effective to control warble flies on cattle; useful against sucking insects, mites and midge larvae; LD_{50}, 330 - 800 mg/kg.

O-ethyl O-methyl O-(2,4,5-trichlorophenyl) phosphorothionate.

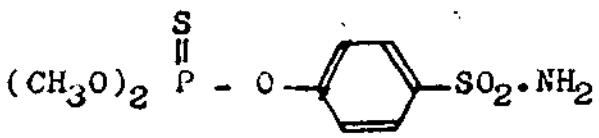
10. Famophos(R), Famphur (American Cyanamid Co.)



Solid, mp. 55°C; systemic insecticide for cattle grubs; LD_{50}, 35 mg/kg.

O,O - dimethyl O - [p-(dimethylsulfamoyl) phenyl]- phosphorothionate.

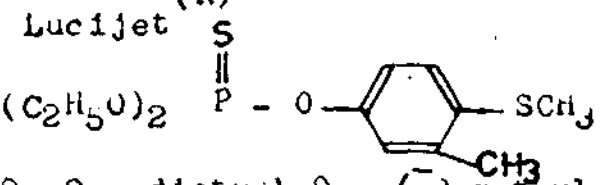
11. Proban(R), Cythioate



Solid, mp. 43-44°C; useful for the control of ticks; LD_{50}, 160 mg/kg.

O,O - dimethyl O [p-(sulfamolyl) phenyl] phosphorothionate.

12. Lucijet(R)

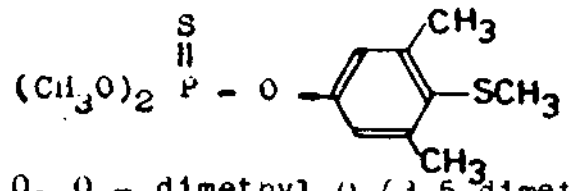


Liquids; insecticide effective against sheep blowfly larvae; LD_{50}, 25 to 100 mg/kg.

O, O - diethyl O - [3-methyl-4-methylthiophenyl]phosphorothionate.

(Contd.)

13. Bay 37342

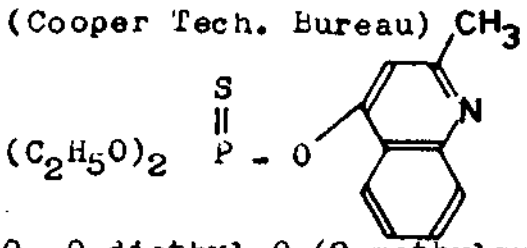


O, O - dimethyl O-(3,5-dimethyl-4-methylthiophenyl) phosphorothionate.

Liquid; animal systemic insecticide for the control of sheep botfly; LD_{50}, 1000 mg/kg.

14. Quinothion

(Cooper Tech. Bureau)



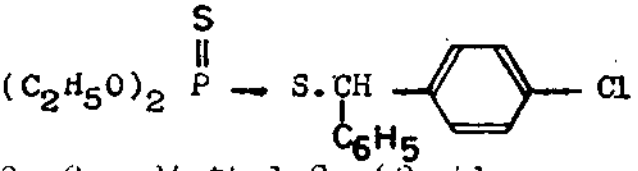
O, O-diethyl O-(2-methylquinolin-4-yl) phosphorothionate.

Liquid; insecticide effective against the sheep parasite <u>Psoroples Communis ovis scab</u>.

C: <u>PHOSPHOROTHIOLOTHIONATES.</u>

15. R - 3,828

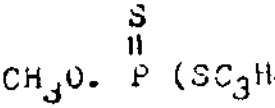
(Stauffer Chem. Co.)



O, O - diethyl S- (P-chloro -phenylbenzyl) phosphorothiolothionate.

Animal systemic insecticide; LD_{50}, 10,000 mg/kg.

16.(a) VC 3-668



S, S - dipropyl O - methyl phosphorothionodithiolate.

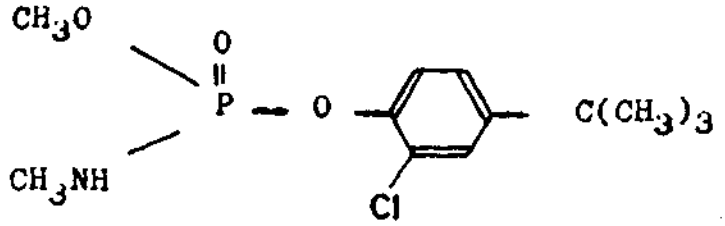
Liquid; effective as a larvicide of manure from chicks by oral administration.

(Contd.)

D: PHOSPHORAMIDATES.

17. Crufomate, Ruelene(R)
(Dow Chem. Co. 1959)

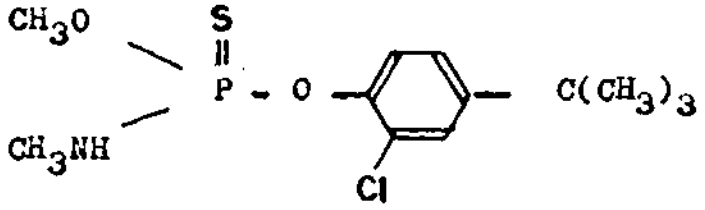
Solid, mp. 60°C; useful for the control of intestinal worms and warble flies etc; LD_{50}, 770 to 1,000 mg/kg.



O - methyl O-(2-chloro-4-tert - butylphenyl) N - methylphosphoramidate.

17. Narlene(R), Dowco 109(R)
(Dow Chem. Co. 1958)

Solid; effective to control cattle grubs and botflies on livestock and also as an anthelmintic; LD_{50}, 1000 mg/kg.



O - methyl O - (2-chloro - 4 - tert-butylphenyl) N-methylphosphoramidothionate.

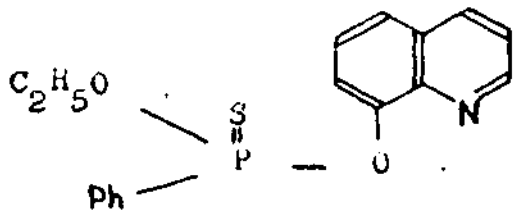
E: PHOSPHONATES.

18. CELA K - 159
O - propyl O-(4-bromo - 2,5-dichlorophenyl) methylphosphonate.

Animal systemic insecticide for the control of cattle grubs; LD_{50},

19. Bacdip(R)

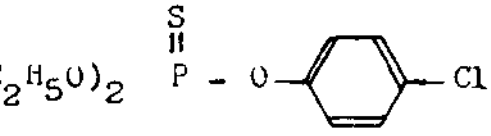
(Bayer AG.)



O- ethyl O-(quinolin-8-yl) phenylphosphorothionate.

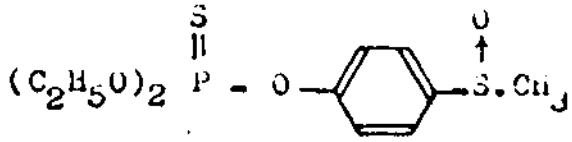
Acaricide; can be used to control tricks on livestock; LD_{50}, 150 mg/kg.

NEMATICIDES

Common or trade name, Chemical name and Chemical designations.	Physical and biological properties.
1. Nemacide(R), Dichlofenthion, VC - 13. (Virginia - Carolina Chem. Corp.)  O, O - diethyl O-(2,4-dichlorophenyl) phosphorothionate.	Liquid; systemic, persistence nemalicide and soil insecticide; LD_{50}, 270 mg/kg.

(Contd.)

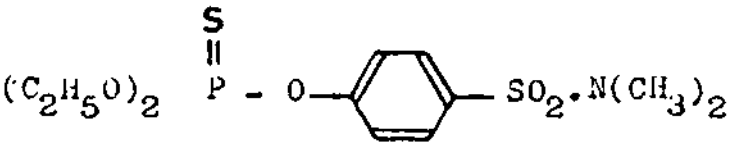
2. Dasanit(R), Fensulfothion, Terracur P(R)



O, O - diethyl O-(p-methylsulfinylphenyl)-phosphorothionate.

Liquid; systemic, persistent nematicide and soil insecticide; it has also contact activity; LD_{50}, 4 to 10 mg/kg.

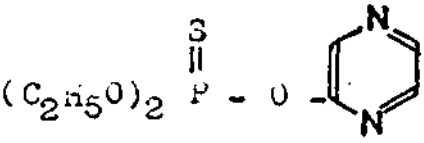
3. DSP; Kaya - ace(R) (Nippon Kayaku Co. 1966)



O, O - diethyl O - (p-dimethylsulfamoylphenyl)-phosphorothionate.

Solid, mp. 68-69°C; persistent nematicide and soil insecticide; LD_{50}, 65.4 mg/kg.

4. Thionazin, Zinophos(R) Nemafos(R), Cynem(R) (American Cyanamid Co. 1966)



O-ethyl S,S - dipropyl phosphorodithiolate.

Liquid; systemic soil insecticide and nematicide; persists about 4 weeks in soil; LD_{50}, 12 mg/kg.

5. Mocap(R), Prophos (Mobil Chemical Co.)

$C_2H_5O \cdot P(SC_3H_7)_2$ (O)

O-ethyl S,S - dipropyl phosphorodithiolate.

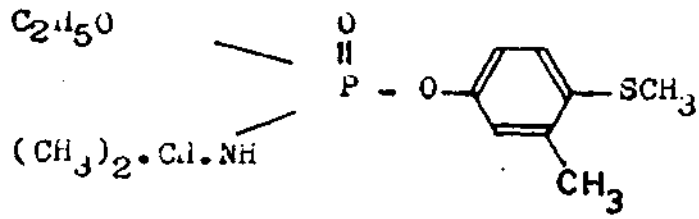
Liquid; contact soil insecticide (corn rootworms) and nematicide; LD_{50}, 61.5 mg/kg.

Other alkylphosphorodithiolate esters:

$Cl\ CH_2 - P(=O)(SC_2H_5)_2$ — Liquid; nematicide.

$CH_3OCH = CH - P(=O)(S - i - C_3H_7)_2$ — Liquid; nematicide.

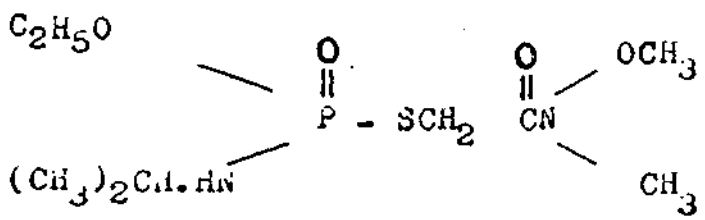
6. Nemacur,(R) Phenamiphos (Bayer AG.) — Solid, mp. 49°C; systemic nematicide, effective against almost all nematode species below 5 ppm in soil for a long time (3 to 4 months).



O - ethyl O-(3-methyl-4-methylthiophenyl) N-isopropylphosphoramidate.

LD_{50}, 15.3 to 19.4 mg/kg.

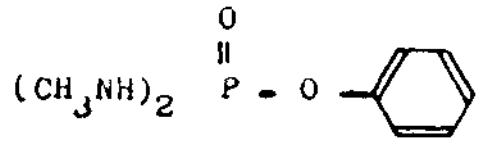
7. FCS 13 — Nematicide.



O - ethyl S - (N - methoxy - N - methylcarbamoylmethyl) N-isopropylphosphoramidothiolate.

(Contd.)

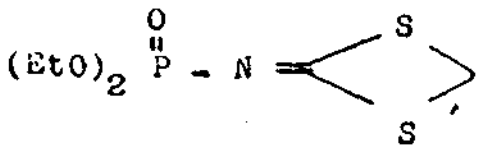
8. Nellite(R), Dowco 189 (Dow Chem. Co.)



O, - phenyl N,N' dimethyl phosphorodiamidate.

Solid, mp. 105-106°C; systemic nematicide, specifically effective against root-knot nematode larvae; LD_{50}, 250 mg/kg.

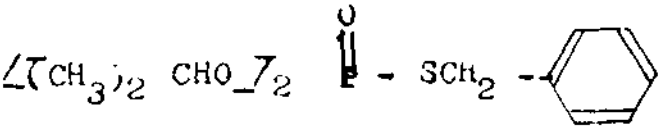
9. Fosthietan, Acconem, Geophos. (American Cynamide Co. 1972)



1,3 - dithietan-2-ylidene-phosphoramidate.

Liquid, broad- range nematicide and insecticide, effective in soil application. LD_{50}, 5.7 mg tech/kg.

FUNGICIDES

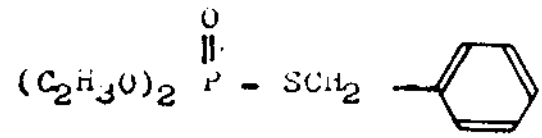
Common or trade name, Chemical name and Chemical designation.	Physical and biological properties.
1. Kitazin P(R), IBP (Kumiai Chem. Co. 1965)  O, O - diisopropyl S-benzylphosphorothiolate.	Liquid; systemic fungicide, inhibits strongly the myceliah growth and spore formation of *Pericularia oryzae*; has also weak insecticidal activity with fenitrothion; LD_{50}, 238 mg/kg (M)

(Contd.)

2. Kitazin(R)

(Kumiai Chem. Co. 1967)

Liquid; systemic fungicide; LD_{50}, 233 mg/kg (M)

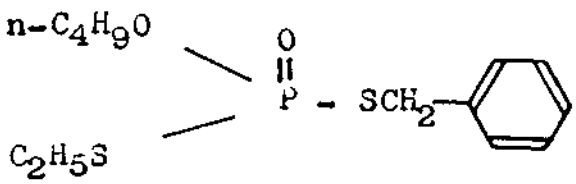


O, O - diethyl S - benzyl phosphorothiolate.

3. Conen(R) BEBP

(Sumitomo Chem. Co.)

Liquid; fungicide to control rice blast disease; LD_{50}, 118 mg/kg.

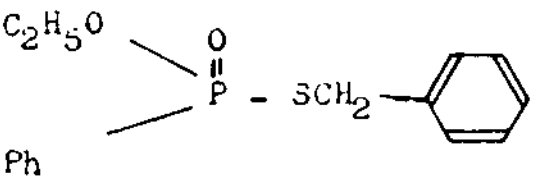


S - Benzyl S-ethyl O-(n-butyl) phosphorodithiolate.

4. Inezin(R), ESBP

(Nissan Chem. Co.)

Liquid; fungicide to control rice blast and rice sheath blight; LD_{50}, 720 mg/kg.

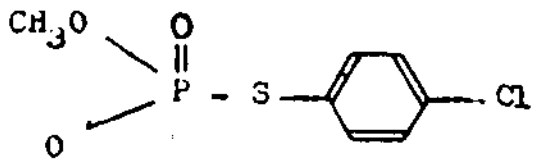


O - ethyl S - benzyl phenylphosphonothiolate.

(Contd.)

5. Cerezin(R)

(Nihon Tukushu Noyaku Seizo Co.1966)



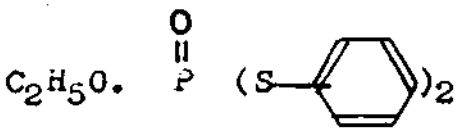
Liquid; has curative effect on rice blast disease and has also insecticidal activity;

LD_{50}, 160 mg/kg.

O - methyl O - Cyclohexyl S - (p-chlorophenyl) phosphorothiolate.

6. Hinosan(R), Edifenphos, EDDP

(Bayer AG; Nihon Tokushu Noyaku Seize Co.)



Liquid; fungicide, effective against rice blast disease; has prophylastic effect against <u>P. Oryzae</u>

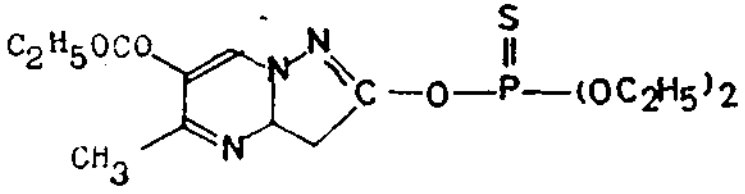
LD_{50}, 140 mg/kg.

O-ethyl S, S-diphenyl phosphorodithiolate.

Remarks: n-propyl and isopropyl homologs have almost the same fungicidal activity as Hinosan, but methyl and butyl homologs are much less active than the latter.

7. Pyrazophos, HOE 2873, Afugan(R)

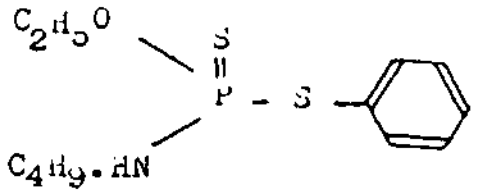
Systemic fungicide, effective against powdery mildews.



O,O- diethyl O-(6-ethoxycorbonyl-5-methylpyrasolol [1,5-a] pyrimidin-2-yl) phosphorothionate.

(Contd.)

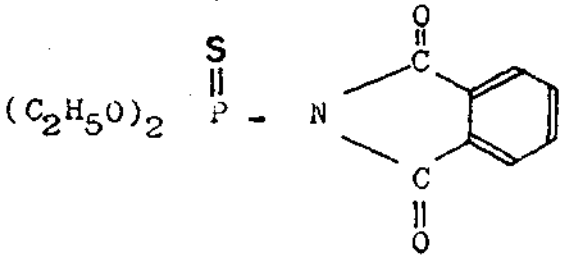
8. Phosbutyl



O - ethyl S-phenyl N-butylphosphoramidothiolothionate.

Liquid; fungicide, effective against mycelial cell formation, but not active against spore germination, LD_{50}, 300 mg/kg.

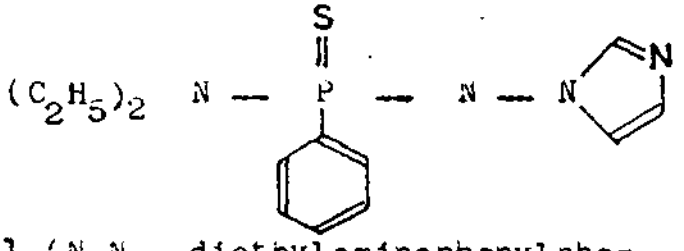
9. Ditalimfos, Dowco 199



O, O - diethyl phthalimidophosphonothionate.

Solid, mp. 83-84°C; fungicide, effective to control powdery mildew, shows curative and protective action; LD_{50}, 5660 mg/kg.

10.

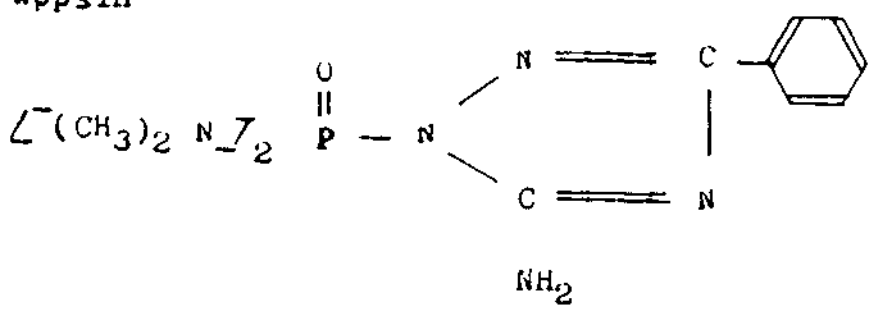


1-(N,N - diethylaminophenylphosphinothioyl) - imidazole.

Solid, mp. 48°C; fungicide, effective against *Erysiphe cichoracearum* on Cucumbers and *Phytophthora infestans* on potato plants; LD_{50}, 1000 mg/kg.

(Contd.)

11. Triamiphos, Wepsyn(R), Wepsin(R)



5-amino-1- [bis-(dimethylamino) phosphinyl]-3-phenyl-1,2,4-triazole 5-anilino-3-alkyl analogs are also active as fungicides and insecticides.

Solid, mp. 167-168°C; insecticide and fungicide, effective against powdery mildew on apples and roses; useful as an acaricide with systemic properties; LD_{50}, 20 mg/kg.

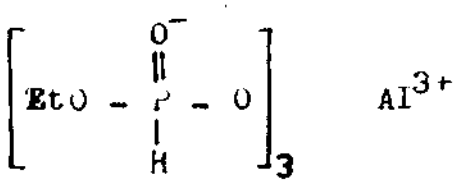
12. Decafentin

$[(C_6H_5)_3 \overset{+}{P} C_{10}H_{21}]\ [(C_6H_5)_3 Sn Br \overset{-}{Cl}]$

Fungicide, LD_{50}, 450 - 515 mg/kg.

13. Aliette, Mikal

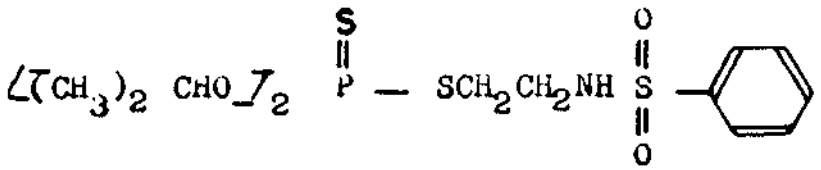
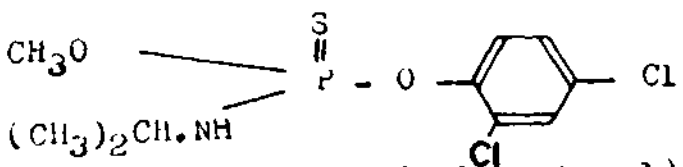
(Rhone - Poulenc Phytosanitare, 1978)



Aluminium tris (ethyl phosphonate).

Colourless powder, decomposing 200°C, systemic fungicide, effective against phycomycete diseases LD_{50}, 5800 mg a-i/kg.

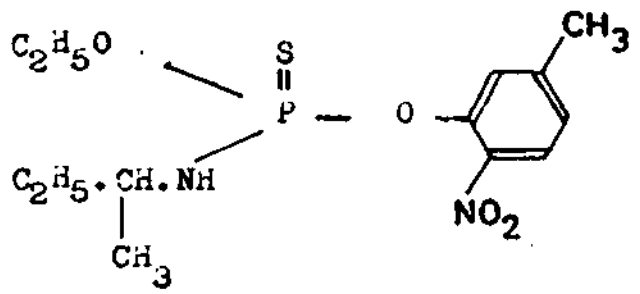
HERBICIDES AND PLANT GROWTH REGULATORS

Common or Trade name Chemical name and Chemical designations.	Physical and biological properties
1. Prefar(R), Betasan(R), Bensulide (Stauffer Chem. Co. 1964)  N-(2-mercaptoethyl) benzenesulfonamides-S-(O,O-- di-isopropyl phosphorodithionate).	Super cooled liquid or solid, mp. 34.5; herbicide, effective for the pre-emergence control of weeds, principally crabgrans, with a long action, LD_{50}, 770 mg/kg.
2. DEF(R), bulifos (Ethyl Corporation. 1954). $(C_4H_9S)_3\ P = O$ S,S,S - tributyl phosphorotrithiolate.	Liquid, effective defoliant for use on cotton; it is a synergist of malathion; LD_{50}, 325 mg/kg.
3. DMPA, Zytron(R) (Dow Chem. Co. 1959).  O-methyl O-(2,4 - dichlorophenyl) N-isopropylphosphoramidothionate.	Solid, mp. 51°C, pre-emergence selective herbicide for control of Crabgran in turf; LD_{50}, 270 mg/kg, it shows delayed neurotoxicity.

(Contd..)

4. Cremart,
S - 2846
(Sumitomo Chem. Co.)

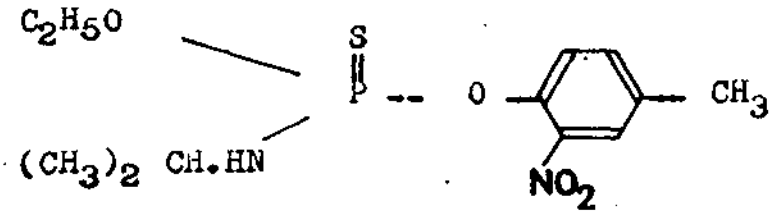
Liquid; pre-emergence harbicide, used in Cereal, beans, Carrot etc.; LD_{50}, 790 mg/kg (male rat).



O - ethyl O-(3-methyl-6-nitrophenyl) N-sec - butylphosphoramidothionate.

5. Amiprophos
(Nihon Tokushu Noyaku Co. 1971)

herbicide; LD_{50}, 720 mg/kg.



O - ethyl O-(2-nitro-4-methyl) N-isopropyl Phosphoramidothionate.

6. Ethephon, Ethrel (R)
(Amchem Products Incorporated)

$Cl - CH_2CH_2 \overset{O}{\overset{\|}{P}} (OH)_2$

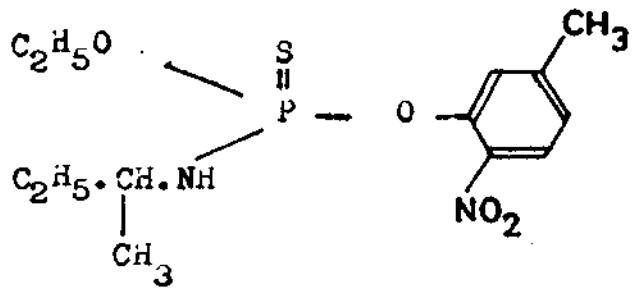
2-chloroethylphosphonic acid

Hygroscopic solid, mp. 74-75°C; plant growth regulator; LD_{50}, 4,200 mg/kg.

(Contd..)

4. Cremart,
S - 2846
(Sumitomo Chem. Co.)

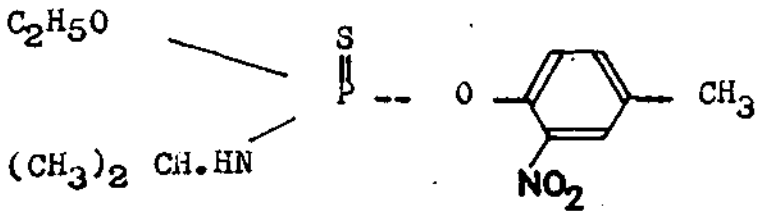
Liquid; pre-emergence harbicide, used in Cereal, beans, Carrot etc.; LD_{50}, 790 mg/kg (male rat).



O - ethyl O-(3-methyl-6-nitrophenyl) N-sec - butylphosphoramidothionate.

5. Amiprophos
(Nihon Tokushu Noyaku Co. 1971)

herbicide; LD_{50}, 720 mg/kg.



O - ethyl O-(2-nitro-4-methyl) N-isopropyl Phosphoramidothionate.

6. Ethephon, Ethrel (R)
(Amchem Products Incorporated)

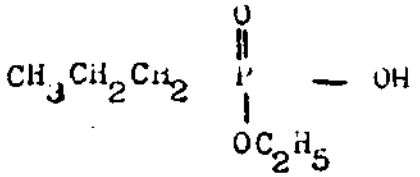
$Cl - CH_2CH_2 \overset{O}{\overset{\|}{P}} (OH)_2$

2-chloroethylphosphonic acid

Hygroscopic solid, mp. 74-75°C; plant growth regulator; LD_{50}, 4,200 mg/kg.

(Contd.)

7. NIA 10637

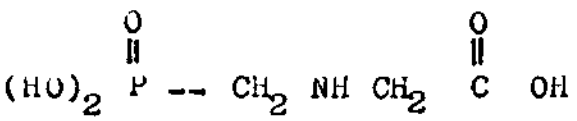


O-ethyl np-propylphosphonic acit.

Plant growth regulator, by reducing internode elongation;

8. Glyphosate

(Monsanto Co. 1971)



N-(phosphonomethyl) glycine

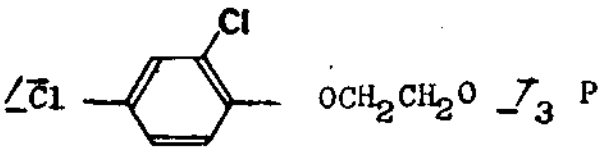
The monp-isoproypylamine salt of Glyphosate is called Roundup(R).

Solid, mp. 230°C; postemergence herbicide for broad spectrum control of both annual and perennial weeds; LD_{50}, 4,320 mg/kg.

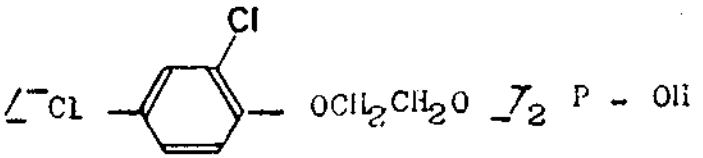
9. Falone(R), Falodin(R)

2,4-DEP

(Uniroyal Incorporated)



(tris form)



(bis form)

Useful for pre-emergence weed control;

LD_{50}, 850 mg/kg.

10. Folex(R),

merphos

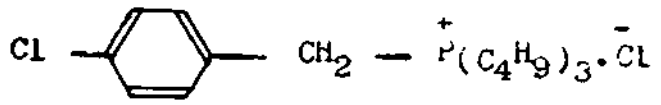
$(C_4H_9S)_3$ P

Tributyl phosphorotrithioite.

Liquid; very effective cotton defoliant; LD_{50}, 1,270 mg/kg.

(Contd.)

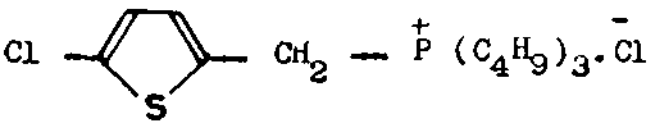
11. Phosfon(R)



2,4-dichlorobenzyltributylphosphonium chloride

Solid, mp. 114 to 120°C; effective as height retardant; LD_{50}, 173 mg/kg.

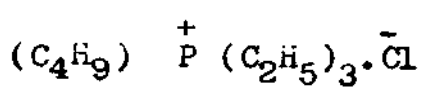
12. CTBP



5-chloro-2-thenyltributylphosphonium-chloride

The yield of snap beans (<u>phaseolus vulgaris</u>) is increased by a foliar spray of CTBP.

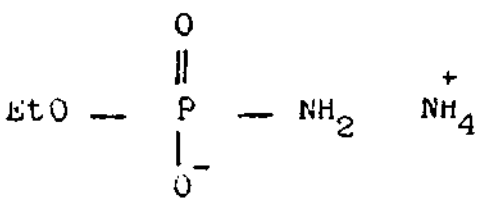
13.



Butyltriethylphosphoniumchloride

Defoliant.

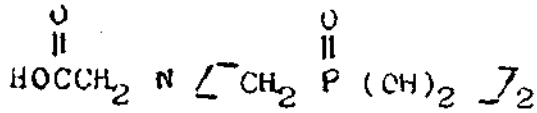
14. Fosamine - amonium



Amonium ethylcarbamoylphosphonate.

Solid, mp. - 175°C effective against woody plants. LD_{50}, 24,000 mg/kg.

15. Glyphosine

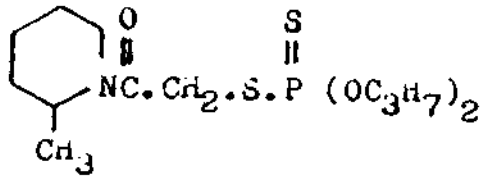


N,N- bis (phosphonomethyl) glycine.

Solid, plant growth regulator used to hasten ripening of sugarcane and to increase sucrose content.
LD_{50}, 3926 mg/kg.

16. Piperophos.

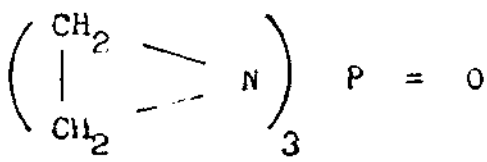
(Ciba - Geigy AG. 1969)



S-2-methyl piperidino carbonyl methyl
O, O - dipropyl phosphorodithioate.

Oil in room temp., but decomposes on heating, selective herbicide against annual grasses and sedges in rice, LD_{50}, 324 mg/kg.

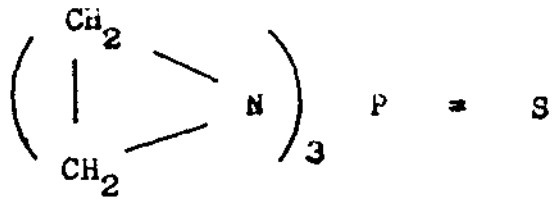
INSECT CHEMOSTERILANTS

Common or trade name, Chemical name and Chemical designations.	Physical and biological properties.
1. Tepa, APO, Aphoxide  Tris-(1-aziridinyl) phosphine oxide.	Hygroscopic solid, mp.45°C, highly selective sterilant, LD_{50}, 37 mg/kg.

(Contd.)

2. Thiotepa

Solid, mp. 51.5° ; suitable for sterilization of Culex pipiens quinquefasciatus.

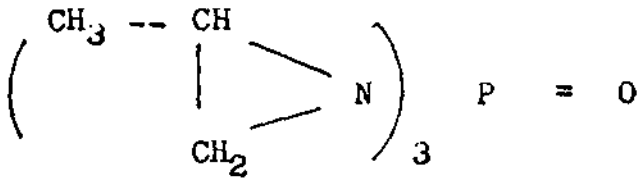


Tris - (1-aziridinyl) phosphine sulphide

3. Metapa, MAPO

Methaphoxide.

Liquid; LD_{50}, 136 mg/kg.



Tris (2-methyl-1-aziridinyl) phosphine oxide

4. Hempa,

HMPA, HMP

Liquid; LD_{50}, 2640 mg/kg.

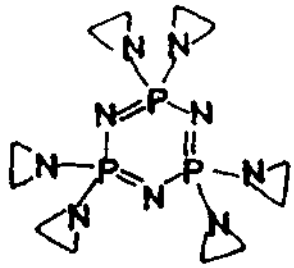
$[(CH_3)_2 N]_3 P = O$

Hexamethylphosphoric triamide

(Contd.)

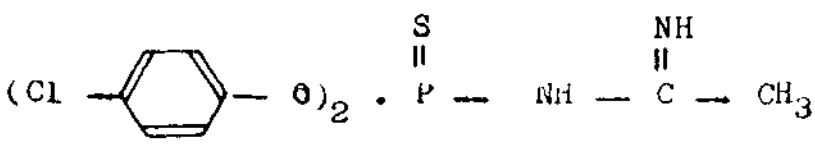
5. Apholate, Omaflora(R) — Solid, mp. 155°C.

APN



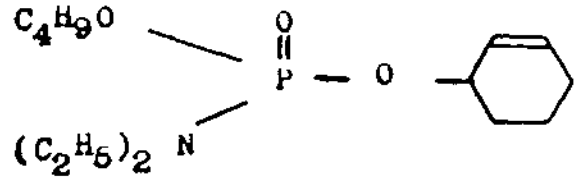
2,2,4,4,6,6 - Hexakis (1-aziridinyl)-2,2,4,4,6,6 - Hexahydro - 1,3,5,2,4,6-triazatriphosphorine.

RODENTICIDES, INSECT RAPALLENTS AND INSECTICIDE SYNERGISTS.

Common or trade name, Chemical name and Chemical designations.	Physical and biological properties.
1. Gophacide(R) (Bayer AG)  O,O - di-(p-chlorophenyl) N-acetimidophosphoramidothionate.	Solid, mp. 104-106°C; rodenticide; LD_{50}, 7.5 mg/kg.

(Contd.)

2. Liquid; repellents for fleas, mosquitoes and house flies.



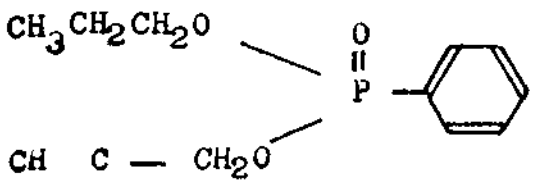
O-butyl O-cychohexenyl N,N-diethylphosphoramidate.

n-butyl isomer, b.p. 173-175°C (9 mm Hg)

Iso " " , b.p. 165-170°C (8 mg Hg)

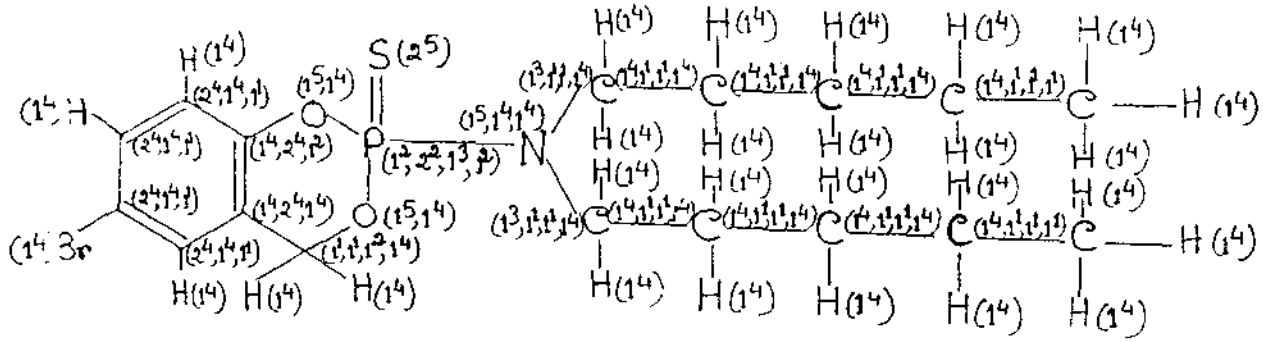
3. NIA 16388 (Niagara Chemical Division FMC)

Liquid, synergist of pyrethroids; shows juvenile hormone activity; LD_{50}, 362 mg/kg.



O - n - propyl O - (2-propynyl) phenyl phosphonate.

APPENDIX - II



BR-8

$n = 50$

H : $\{(1^4) \times 27\}$

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

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

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

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

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

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

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

$\{1^4, 2^4, 1^2) \times 1\}$; $\{(1^4, 1^1, 1^1, 1^4) \times 6\}$

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

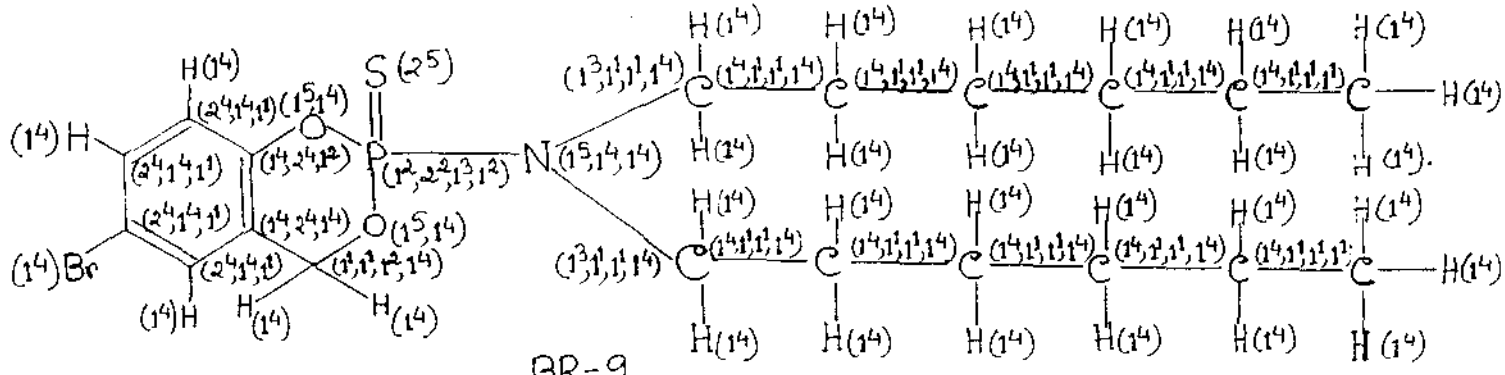
$$I'(C) = -\left\{1 \times \frac{27}{50} \log_2 \frac{27}{50} + 3 \times \frac{2}{50} \log_2 \frac{2}{50} + 1 \times \frac{4}{50} \log_2 \frac{4}{50} + 1 \times \frac{6}{50} \log_2 \frac{6}{50} + 7 \times \frac{1}{50} \log_2 \frac{1}{50}\right\}$$

$$= -\left\{\frac{27}{50} \log_2 27 + \frac{6}{50} \log_2 2 + \frac{4}{50} \log_2 4 + \frac{6}{50} \log_2 6 + \log_2 \frac{1}{50}\right\}$$

$$= 2.4864 \text{ bits}$$

$$I(C) = -\left\{50 \times \frac{1}{50} \log_2 \frac{1}{50}\right\} = 5.6445 \text{ bits}$$

$$S.I.C = 2.4864 / \log_2 50 = 0.4405$$



BR-9

$n = 56$

H: $\{(1^4) \times 31\}$

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

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

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

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

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

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

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

$\{(1^4, 2^4, 1^2) \times 1\}$; $\{(1^4, 1^1, 1^1, 1^4) \times 8\}$

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

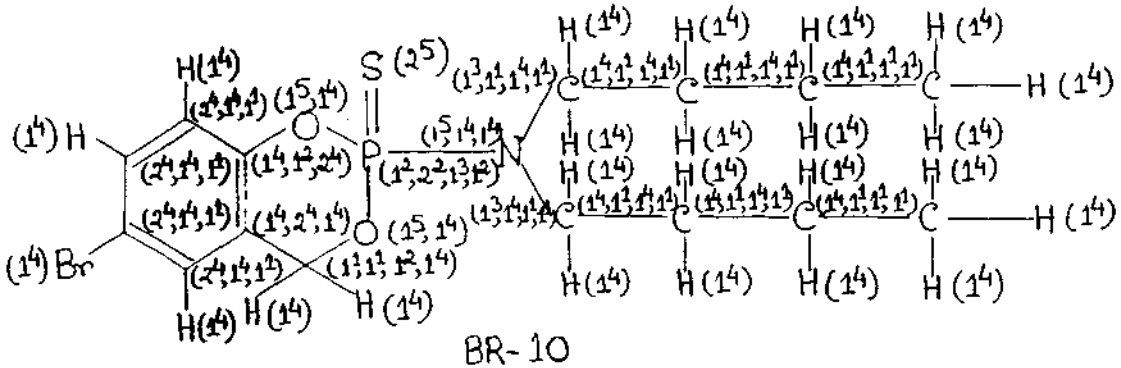
$$I'(C) = -\left\{1 \times \frac{31}{56} \log_2 \frac{31}{56} + 3 \times \frac{2}{56} \log_2 \frac{2}{56} + 1 \times \frac{4}{56} \log_2 \frac{4}{56} + 1 \times \frac{8}{56} \log_2 \frac{8}{56} + 7 \times \frac{1}{56} \log_2 \frac{1}{56}\right\}$$

$$= -\left\{\frac{31}{56} \log_2 31 + \frac{6}{56} \log_2 2 + \frac{4}{56} \log_2 4 + \frac{8}{56} \log_2 8 + \log_2 \frac{1}{56}\right\}$$

$$= 2{\cdot}3867 \text{ bits}$$

$$I(C) = -\left\{56 \times \frac{1}{56} \log_2 \frac{1}{56}\right\} = 5{\cdot}8080 \text{ bits}$$

$$S.I.C = 2{\cdot}3867 / \log_2 56 = 0{\cdot}4109$$



BR-10

$n = 44$

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

H: $\{(1^4)\times 23\}$

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

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

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

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

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

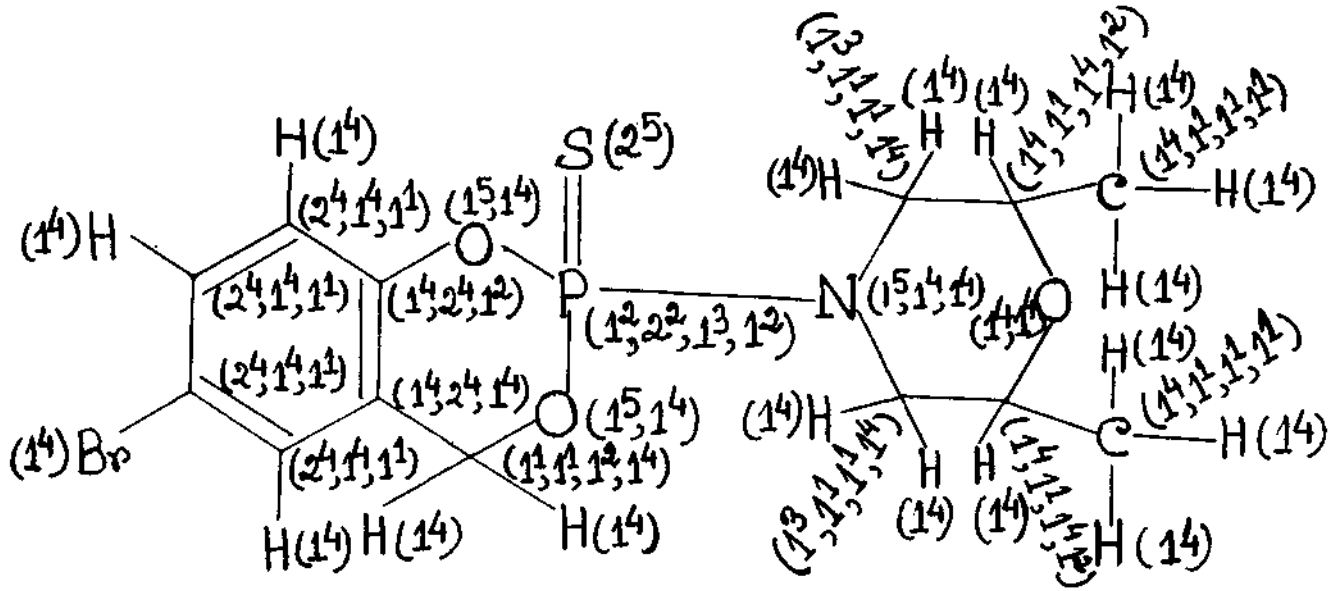
$$I'(C) = -\left\{1\times\frac{23}{44}\log_2\frac{23}{44} + 3\times\frac{2}{44}\log_2\frac{2}{44} + 2\times\frac{4}{44}\log_2\frac{4}{44} + 7\times\frac{1}{44}\log_2\frac{1}{44}\right\}$$

$$= -\left\{\frac{23}{44}\log_2 23 + \frac{6}{44}\log_2 2 + \frac{8}{44}\log_2 4 + \log_2\frac{1}{44}\right\}$$

$$= 2.5951 \text{ bits}$$

$$I(C) = -\left\{44\times\frac{1}{44}\log_2\frac{1}{44}\right\} = 5.46 \text{ bits}$$

$$S.I.C = 2.5951/\log_2 44 = 0.4753$$



BR-12

$n = 37$

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

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

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

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

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

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

$\{(1^4, 1^4) \times 1\}$

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

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

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

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

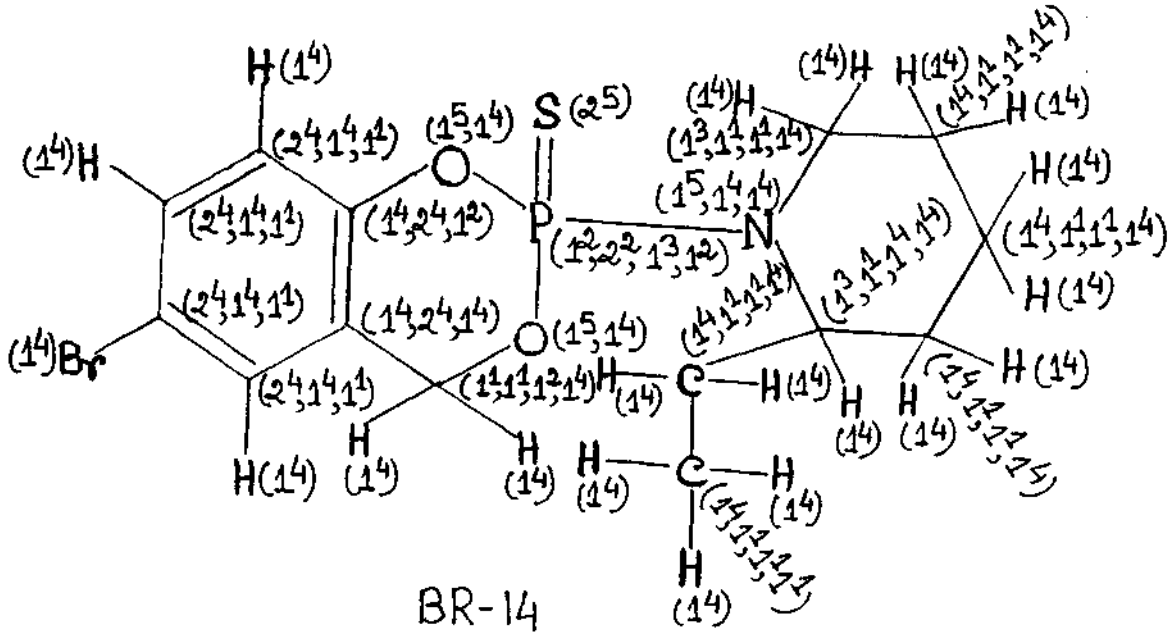
$$I'(C) = -\left\{1 \times \frac{17}{37} \log_2 \frac{17}{37} + 4 \times \frac{2}{37} \log_2 \frac{2}{37} + 1 \times \frac{4}{37} \log_2 \frac{4}{37} + 8 \times \frac{1}{37} \log_2 \frac{1}{37}\right\}$$

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

$$= 2.8994 \text{ bits}$$

$$I(C) = -\left\{37 \times \frac{1}{37} \log_2 \frac{1}{37}\right\} = 5.21 \text{ bits}$$

$$S.I.C = 2.8994 / \log_2 37 = 0.5565$$



BR-14

$n = 39$

H: $\{(1^4) \times 19\}$

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

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

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

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

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

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

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

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

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

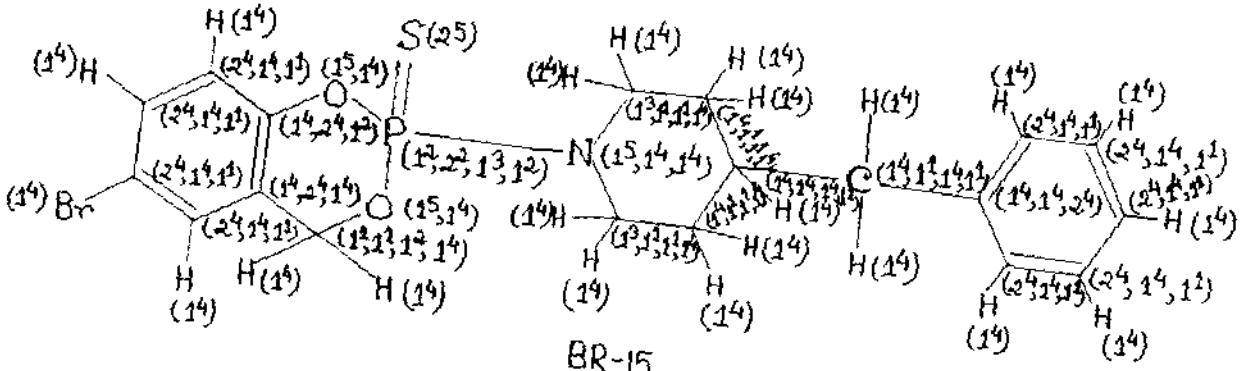
$$I'(C) = -\left\{1 \times \frac{19}{39} \log_2 \frac{19}{39} + 1 \times \frac{2}{39} \log_2 \frac{2}{39} + 2 \times \frac{4}{39} \log_2 \frac{4}{39} + 10 \times \frac{1}{39} \log_2 \frac{1}{39}\right\}$$

$$= -\left\{\frac{19}{39} \log_2 19 + \frac{2}{39} \log_2 2 + \frac{8}{39} \log_2 4 + \log_2 \frac{1}{39}\right\}$$

$= 2{\cdot}7548$ bits

$$I(C) = -\left\{39 \times \frac{1}{39} \log_2 \frac{1}{39}\right\} = 5{\cdot}2860 \text{ bits}$$

$$S.I.C = 2{\cdot}7548 / \log_2 39 = 0{\cdot}5211$$



BR-15

$n = 46$

H: $\{(1^4) \times 21\}$

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

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

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

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

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

C: $\{(2^4, 1^4, 1^1) \times 9\}$; $\{(1^3, 1^1, 1^1, 1^4) \times 2\}$
$\{(1^4, 2^4, 1^4) \times 1\}$; $\{(1^4, 1^1, 1^1, 1^4) \times 3\}$
$\{(1^4, 2^4, 1^2) \times 1\}$; $\{(1^4, 1^4, 1^4, 1^1) \times 1\}$
$\{(1^1, 1^1, 1^2, 1^4) \times 1\}$; $\{(1^4, 1^4, 2^4) \times 1\}$

$$I'(C) = -\left\{1 \times \frac{21}{46} \log_2 \frac{21}{46} + 2 \times \frac{2}{46} \log_2 \frac{2}{46} + 1 \times \frac{3}{46} \log_2 \frac{3}{46} + 1 \times \frac{9}{46} \log_2 \frac{9}{46} + 9 \times \frac{1}{46} \log_2 \frac{1}{46}\right\}$$

$$= -\left\{\frac{21}{46} \log_2 21 + \frac{4}{46} \log_2 2 + \frac{3}{46} \log_2 3 + \frac{9}{46} \log_2 9 + \log_2 \frac{1}{46}\right\}$$

$$= 2{\cdot}7082 \text{ bits}$$

$$I(C) = -\left\{46 \times \frac{1}{46} \log_2 \frac{1}{46}\right\} = 5{\cdot}5242 \text{ bits}$$

$$S.I.C = 2{\cdot}7082 / \log_2 46 = 0{\cdot}4902$$

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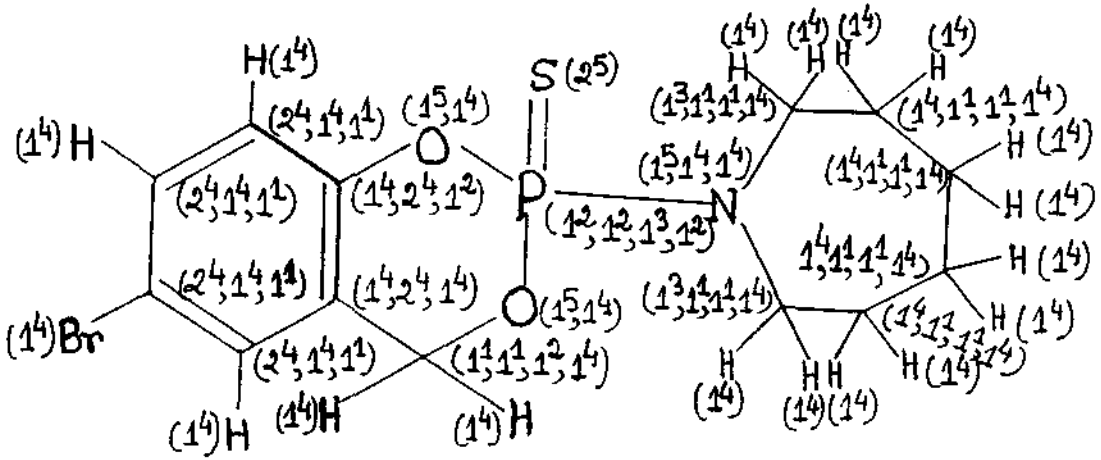

BR-17

$n = 36$

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

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

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

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

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

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

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

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

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

$$I'(C) = -\left\{ 1 \times \frac{17}{36} \log_2 \frac{17}{36} + 2 \times \frac{2}{36} \log_2 \frac{2}{36} + 2 \times \frac{4}{36} \log_2 \frac{4}{36} + 7 \times \frac{1}{36} \log_2 \frac{1}{36} \right\}$$

$$= -\left\{ \frac{17}{36} \log_2 17 + \frac{4}{36} \log_2 2 + \frac{8}{36} \log_2 4 + \log_2 \frac{1}{36} \right\}$$

$$= 2{\cdot}6846 \text{ bits}$$

$$I(C) = -\left\{ 36 \times \frac{1}{36} \log_2 \frac{1}{36} \right\} = 5{\cdot}1705 \text{ bits}$$

$$S.I.C = 2{\cdot}6846 / \log_2 36 = 0{\cdot}5192$$