PART III

PART - III

STUDIES ON SOME ORGANOPHOSPHORUS COMPOUNDS : SPECTRAL PROPERTIES, BIOLOGICAL ACTIVITIES AND HYDROLYTIC PROPERTIES.

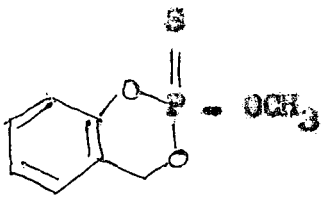
(2-alkylamido-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-sulphide).

[Synthesis, spectral properties (^{1}H -, ^{13}C - and ^{31}P - NMR, UV, IR, and mass spectral), biological activities (insecticidal, fungicidal, and toxicological activities including antichôlinesterase properties), and hydrolytic properties of some 2-alkylamido-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-sulphides].

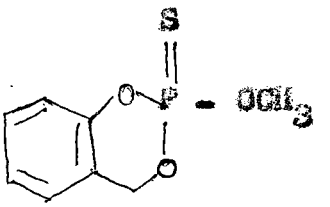
PART - III

AIMS AND OBJECTS OF THE PRESENT INVESTIGATION:

As stated previously (in PART - II) salithion (2-methoxy-4H-1,3,2-benzodioxaphosphorin-2-sulphide) was discovered in 1963 in the Laboratory of Pesticide Chemistry, Kyushu University, JAPAN[1], and developed into commercialization in 1968 by Sumitomo Chemical Company; many related compounds have been synthesized to study their chemical, biochemical, hydrolytic and biological properties including pesticidal activities. The unique structure of salithion comes from the active metabolite of tri-ortho-tolyl phosphate (TOCP)[2]. In spite of high neurotoxicity of the TOCP-metabolite, salithion causes no such toxicity. The introduction of any type of substituent at any position of the benzene ring decreases the insecticidal activity[3]. It has been reported[3] 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 compared to salithion[3]. However, it has been observed[4] in this laboratory that the methoxy compound (BD-8) is a white crystalline solid (m.p. 84°C), and has about 1.5-2 times greater oral insecticidal activity to cockroaches, Periplaneta americana (Linn) compared to salithion.

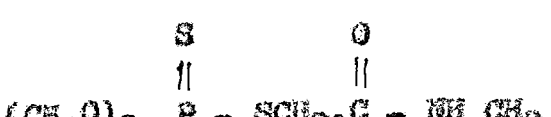


Salithion

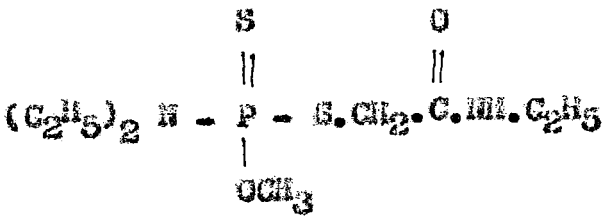


BD - 8 (m.p.-84°C)

Moreover, introduction of an amide group in place of an alkyl ester group often affords organophosphorus esters with fungicidal activity[5]. For example, although the insecticide dimethoate(I) [dimethyl S-(N-methylcarbamoylmethyl) phosphorothiolothionate] has no fungicidal activity, its dialkylphosphoramidate analogs, such as compound II, show some fungicidal as well as acaricidal activity[6].



I
Dimethoate

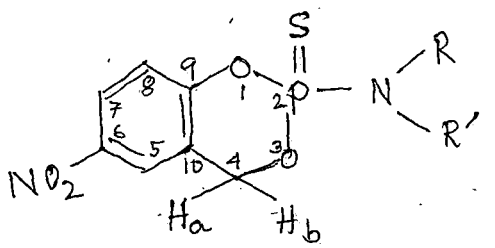


II
Compound- II

There are several other examples in literature from which it can be found that some phosphoramidothionates, phosphoramidothiolothionates, phosphoramides or phosphonamides in which the

phosphorus atom is attached directly to the nitrogen atom of an amine or a heterocyclic compound such as phthalimide, imidazole or triazole have very good fungicidal activity[5,7,8,9].

These observations prompted us to undertake a systematic work on some 2-alkylamido-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-sulphides having general structure (A),



(A)

where, alkylamido is cyclohexylamido, morpholino, dimethylamido, diethylamido, pyrrolidino, piperidino, isopropylamido or nonylamido. The work embodied in this dissertation is related to the investigation of the above mentioned compounds with reference to their chemical, pesticidal and toxicological properties besides structural elucidations by spectroscopic methods.

Actual Works:

(1) Syntheses of some organophosphorus compounds having general structure (A) mentioned above, are presented.

(ii) Spectral Properties: UV, IR, Mass and ^{1}H NMR spectral data of these compounds have been presented; ^{13}C and ^{31}P NMR spectra of only pyrrolidino and piperidino compounds as well as low temperature ^{1}H NMR spectral data of the methoxy compound (BD-8) have been given.

(iii) Biological Activities : Insecticidal, antifungal, anticholinesterase activities and phytotoxic properties have been studied; acute oral toxicity data on rats have also been presented.

(iv) Hydrolytic Properties: Chemical hydrolysis at alkaline pH have been given.

R E F E R E N C E S:

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

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6. Mandelbaum, Ya. A., Soifer, R.S., Mel'nikov, N.N., and Fedoseenko, L.G. : Khim - Sel'sk. Khoz., 6, 107 (1968)
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9. Tolkmith, H. : Nature, 211 (5048), 522 (1966) Science, 155, 85(1967).

A. MATERIALS AND METHODS

1. PURIFICATION OF SOLVENTS AND OTHER CHEMICALS:

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

2. SPECTROSCOPIC METHODS:

Infrared spectra were recorded on Beckmann IR-20 spectrophoto meter in nujol mull. ^{1}H NMR spectra were obtained with Varian model A-60, EM-390, FT-80A, and WH-270 instruments in $CDCl_3$/Acetone - d_6 solvent using TMS as an internal reference; ^{13}C NMR spectra were recorded in the same FT-80A instrument at 20 MH_z; ^{31}P NMR spectra were obtained with FT-80A instrument at 32.2 MH_z, ^{13}C NMR spectra of nitro saligenin has been taken on WH-270 instrument at 67.89 MH_z low temperature ^{1}H NMR Spectra of the methoxy compound were taken on WH-270 instrument at 270 MH_z. UV spectra were obtained with Beckmann DU-2 spectrophotometer in absolute ethanol. Mass spectra were recorded on Varian model EM-600 and MS-30 instrument.

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

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

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

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

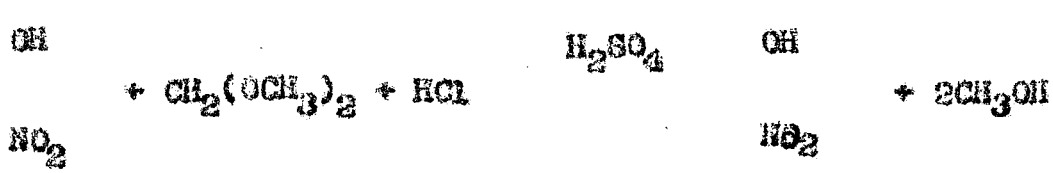
Preparation of the alcohol was done in two stages:

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

(ii) the hydrolysis of the said chloride.

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

The 2-hydroxy-5-nitro-benzyl chloride was prepared according to the method described in Organic Synthesis[3]. The reaction involved is:



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

760 ml. methanol was added to 400 gm anhydrous calcium chloride in a 3 lt. round bottomed flask equipped with a reflux condenser, 10.2 ml. Conc. hydrochloric acid was added; then with cooling and constant stirring, 400 gm of 37-40% formaldehyde was slowly dropped in through a dropping funnel. It took about 2 hours for complete addition of formaldehyde (the reaction was strongly exothermic). When all the formaldehyde had been added, the mixture was heated for a few minutes until the liquid boiled vigorously. The methylal came up quickly on the upper layer, and after an overnight standing was fractionally distilled. The 42-45°C fraction was collected and stored in a tightly stoppered bottle in cold (freezer) before it was used.

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

40-45 gm. of a white product, m.p. 129-130°C.

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

The said chloride was taken as suspension in water and heated slowly; the crystals would go into solution while the impurities form a deep brown oily layer at the bottom of the container. The mixture was then boiled gently to ensure complete hydrolysis. The solution was quickly filtered while hot. The light yellow crystals of 2-hydroxy-5-nitrobenzyl alcohol separated as the filtrate got cooled; the product (m.p. 122-126°C) thus obtained was recrystallised from hot water; the crystals were filtered, washed with cold dioxen : benzene (1:9) mixture, and dried in vacuum.

m.p. 128°C (literature m.p. 128-129°C)[4];

R_f 0.74 (in methanol).

Analysis:

Found ; C, 49.68% ; H, 4.12% ; N, 8.25%;

Calc. for $C_7H_7O_4N$; C, 49.7% ; H, 4.14%; N, 8.28%

UV (Fig. 1) : λ_{max}^{EtOH} = 230 nm (7005), 318(8337), and 410 (3226) nm;

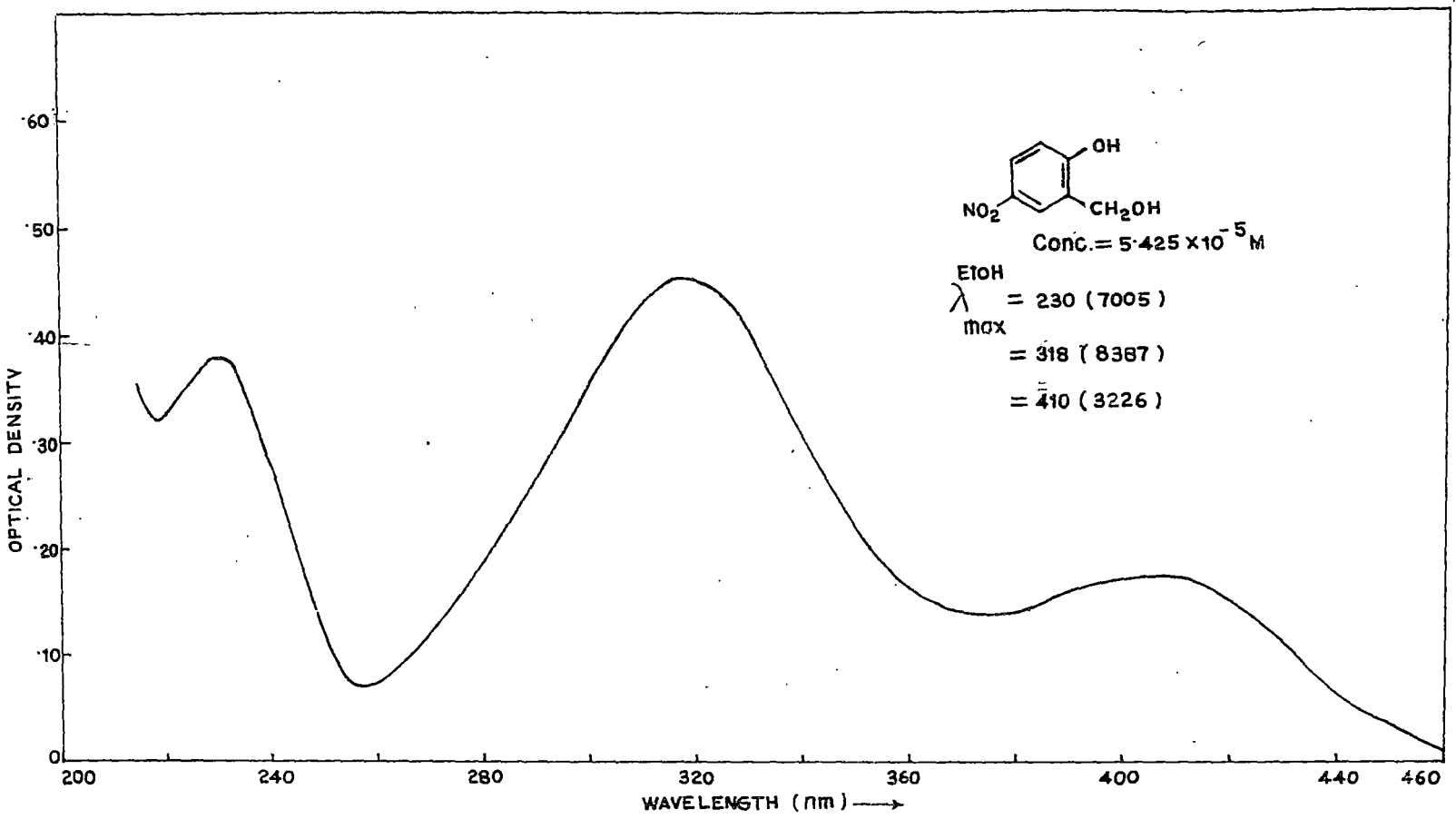
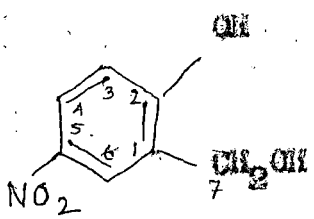


Fig. 1 UV– Visible Spectrum of 5– Nitro saligenin in Ethanol.

IR (Fig.2):

3450 and 3100 Cm^{-1}	: OH vibration;
1438 Cm^{-1}	: OH deformation Str. vibration;
1475 and 1335 Cm^{-1}	: asym. & sym. Str. of NO_2 group;
1610 and 1585 Cm^{-1}	: C = C Str;
1080 Cm^{-1}	: C - C - O
880, 860 and 825 Cm^{-1}	: 1 : 2 : 4 trisubstituted benzene ring vibration;
900 Cm^{-1}	: lone H- atom wagging of the phenyl ring;
750 and 735 Cm^{-1}	: C - N - O bending;
810 Cm^{-1}	: 2H (adjacent) of the ring.

^{13}C NMR (Fig.3):



Carbon Atom	(ppm)
C_7	60.18
C_3	116.02
C_6	124.01
C_1	124.91
C_4	130.41
C_5	141.89
C_2	161.47

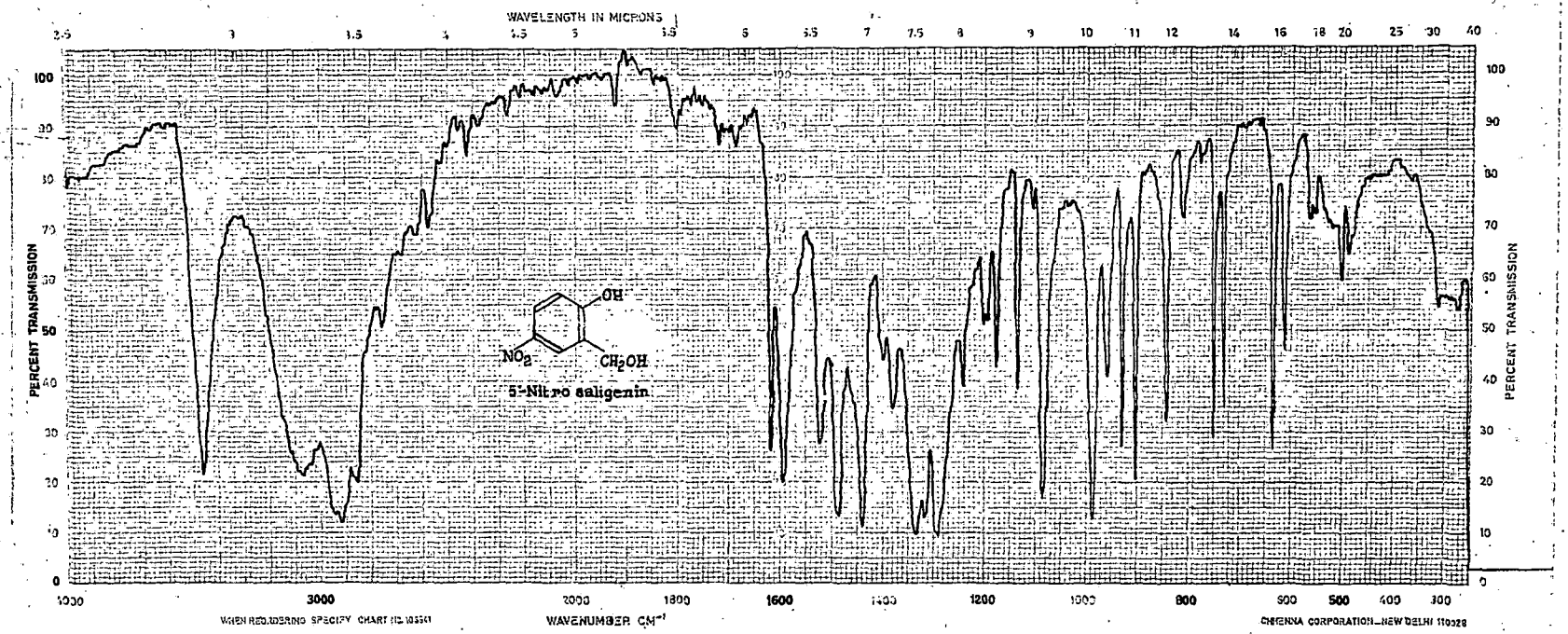


Fig. 2. IR spectrum of 5-Nitro-saligenin

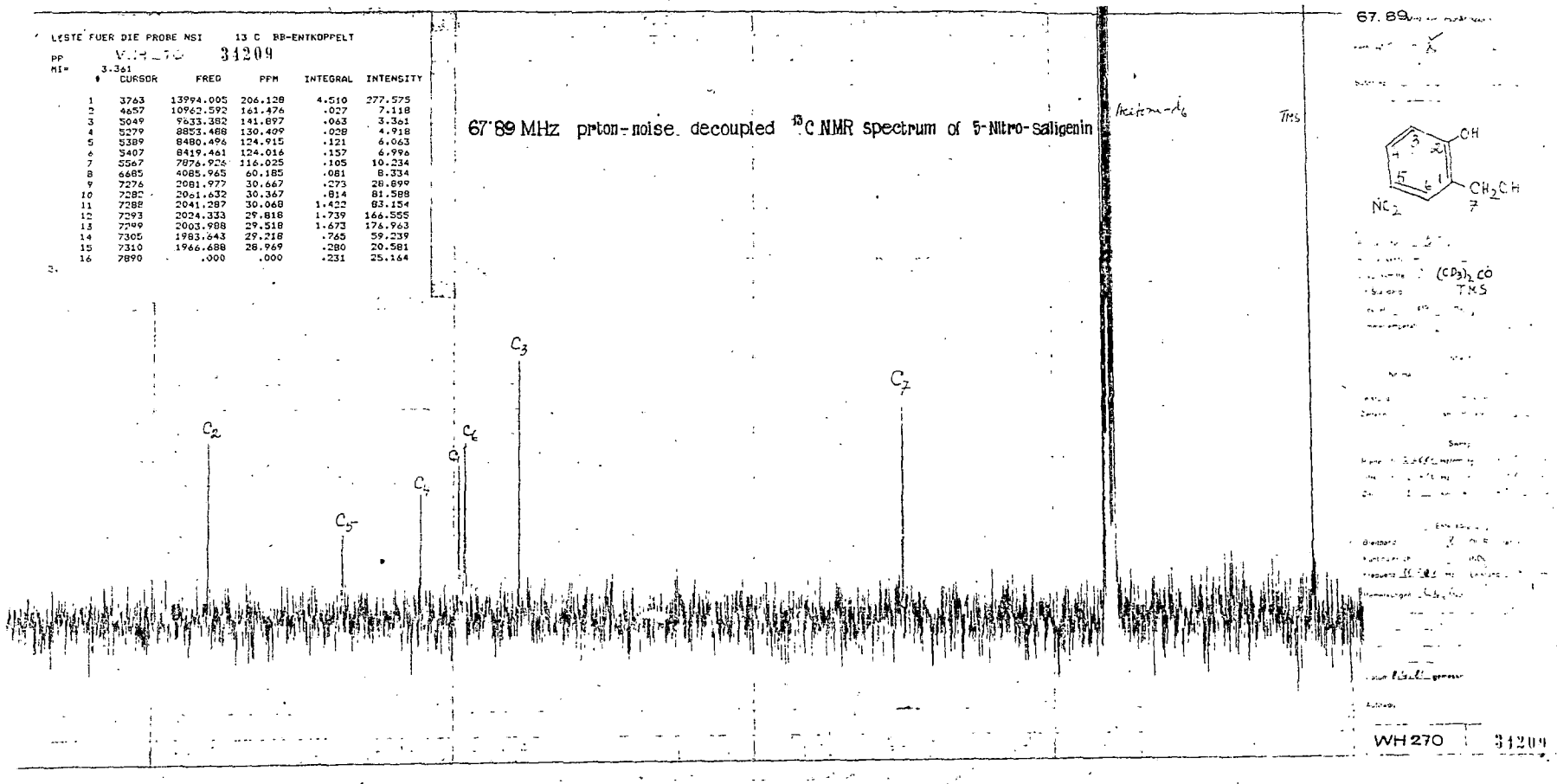

LESTE FUER DIE PROBE NSI 13 C BB-ENTKOPPELT
PP WH270 31209
MI= 3.361
# CURSOR FREQ PPM INTEGRAL INTENSITY
1 3763 13994.005 206.128 4.510 277.575
2 4657 10962.592 161.476 .027 7.118
3 5049 9633.382 141.897 .063 3.361
4 5279 8853.488 130.409 .028 4.918
5 5389 8480.496 124.915 .121 6.063
6 5407 8419.461 124.016 .157 6.996
7 5567 7876.926 116.025 .105 10.234
8 6685 4085.965 60.185 .081 8.334
9 7276 2081.977 30.667 .273 28.899
10 7280 2061.632 30.367 .814 81.588
11 7288 2041.287 30.068 1.422 83.154
12 7293 2024.333 29.818 1.739 166.555
13 7299 2003.988 29.518 1.673 176.963
14 7305 1983.643 29.218 .765 59.239
15 7310 1966.688 28.969 .280 20.581
16 7890 .000 .000 .231 25.164
67.89 MHz proton-noise decoupled ^{13}C NMR spectrum of 5-Nitro-Saligenin
Acetone-d_6
TMS
C_2
C_5
C_4
C_6
C_1
C_3
C_7
67.89
OH
CH_2CH
NO_2
$(CD_3)_2CO$
TMS
WH270 31209

Fig - 3

5. PREPARATION OF PHOSPHORAMIDOTHIOIC DICHLORIDES:

$$2RNH_2 + PSCl_3 \quad (2 \text{ mole}) \quad (1 \text{ mole}) \qquad RNH.P(S)Cl_2 + RNH_2.HCl$$

or

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

One mole $PSCl_3$ and two moles amine (or, one mole amine and one mole triethylamine) were allowed to react at 15° 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 repeatedly with benzene/chloroform, Subsequently with 2% cold hydrochloric acid, then with cold saturated solution of sodium chloride. The benzene/chloroform phase was then dried with anhydrous sodium sulphate and filtered; evaporation *in Vacuo* gave the desired phosphoramidothioic dichloride; this dichloride was used as such for the subsequent reaction.

The different phosphoramidothioic dichlorides were prepared as follows:

5. (1) Cyclo hexylamidophosphoredichloridothionate (IA):

Thiophosphoryl chloride (16.9 gm; 0.1 mole) in 100 ml benzene was allowed to react with cyclohexylamine (19.8gg 0.2 mole)

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

Analysis:

Found : C, 31.04% ; H, 5.20% ; N, 6.02% ; ($C_6H_{12}Cl_2NPS$)

Calc. for: C, 31.05% ; H, 5.21% ; N, 6.03%.

6. (11) Morpholinophosphorodichloridothionate (IB):

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

Analysis:

Found : C, 23.52% ; H, 3.94% ; N, 6.85% ; ($C_4H_8Cl_2NOPS$)

Calc. for: C, 23.54% ; H, 3.95% ; N, 6.86%.

5.(iii) N, N - Diethylamidophosphorodichloridothionate (IC):

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

7.3 gm (0.1 mole, 50.12 ml. benzene extract solution conc. 0.1466 gm/ml) diethylamine was allowed to react with thiophosphoryl chloride (8.45 gm, 0.05 mole) in 50 ml. benzene at -5^{o} to $+5^{o}$C and the mixture was worked up as in (IA). 8.0 gm (78% yield) liquid possessing camphor-like odour was thus obtained.

5.(iv) N, N - Dimethylamidophosphorodichloridothionate (ID):

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

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

Analysis:

Found: C, 13.48% ; H, 3.39% ; N, 7.86% ;

Calc.for: $C_2H_4Cl_2NPS$; C, 13.50% ; H, 3.40% ; N, 7.87%.

5.(v) Isopropylamidophosphorodichloridothionate (IE):

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

Analysis:

Found : C, 18.74% ; H, 4.18% ; N, 7.27% ;

Calc. for : $C_3H_8Cl_2NPS$: C, 18.76% ; H, 4.20% ; N,7.29%.

5. (vi) Pyrrolidinophosphorodichloridothionate (IF):

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

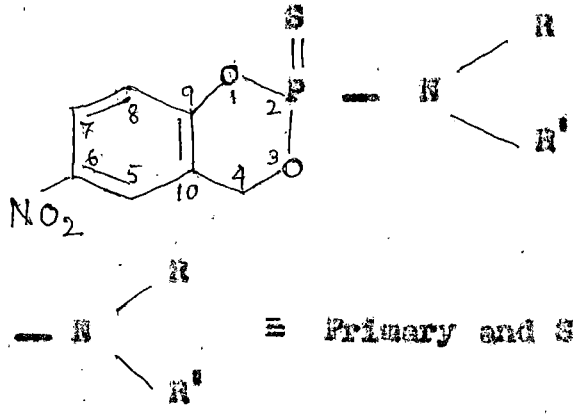
5. (vii). Piperidinophosphorodichloridothionate (IG):

Thiophosphoryl chloride (8.46 gm, 0.05 mole) in 50 ml chloroform was allowed to react with 8.5 gm (0.1 mole) piperidine in 20 ml chloroform. 9.0 gm product was obtained.

5.(viii) Nonylamidophosphorodichloridothionate (III):

This compound was prepared by taking $PSCl_3$ (4.1 gm), triethy-lamine (2.45 gm, 0.0242 mole), and n-nonylamine (3.47 gm, 0.0242 mole) in 100 ml. chloroform; the nonylamine in 30 ml. chloroform was added dropwise; after additional stirring for 3 hrs. the reaction mixture was washed twice with cold saturated sodium chloride solution. The chloroform phase was then dried with anhydrous sodium sulphate and filtered; evaporation in Vacuo gave 6.2 gm liquid nonylamidophosphorodichloridothionate.

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

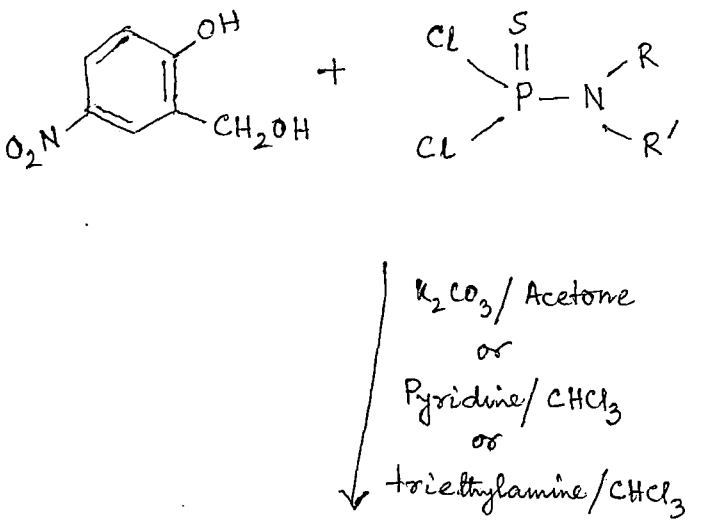


— N(R)(R') ≡ Primary and Secondary amines.

6. (a) GENERAL PROCEDURE:

2-alkylamido-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-Sulphides were prepared by adding a solution of 2-hydroxy-5-nitro benzyl alcohol (5- nitro saligenin, 1 mole) in dry acetone to

1 mole of alkylamidophosphorodichloridothionate with cooling in an ice-bath. The anhydrous potassium carbonate (2 mole) was then added by instalments, with constant stirring. In some cases, good result was found by using 2 moles of pyridine or 2 moles of triethylamine in chloroform solution instead of anhydrous potassium carbonate. The temperature of the reaction mixture was strictly maintained below 5°C during the addition of potassium carbonate/pyridine/triethylamine. The condensation was accomplished by stirring at the temperature 5-27°C for an additional time of 12-16 hrs. The solid particles were filtered out of the reaction mixture and the solvent was removed under reduced pressure at room temperature. In some cases the crude product was directly recrystallised from methanol to give the pure compound; while in others an additional chloroform extraction was necessary prior to the recrystallisation. In the latter case, the crude product was extracted with chloroform and washed with 1% dil HCl (ice - cooled) and with cold water, repeatedly (three times). This was then dried with anhydrous sodium sulphate and the chloroform was subsequently removed under reduced pressure. The pure compounds were then obtained by recrystallisation from methanol.

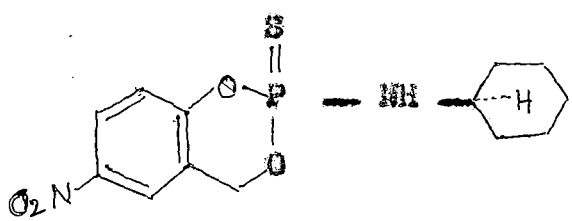


The different alkylamidophosphorothionates were prepared as follows:

6. (b) PREPARATION AND PROPERTIES OF SOME INDIVIDUAL ALKYLAMIDO-PHOSPHOROTHIONATES:

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

6.(b)(i) 2-Cyclohexylamido-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-Sulphide (BD-10):



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

Analysis:

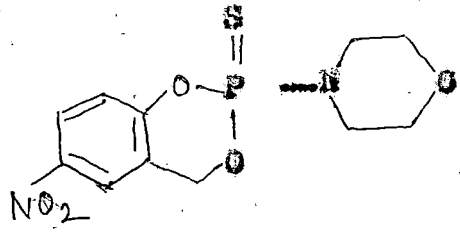
Found : C, 47.54% ; H, 5.19% ; N, 8.52% ;

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

Molecular Weight : 328.

A better yield (95%) was obtained by using pyridine (chloroform as solvent) instead of K_2CO_3 as dehydrogen chloride agent.

6.(b)(ii). 2-Morpholino-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-Sulphide (BD-11):



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

in acetone and chloroform, moderately soluble in methanol, ethanol and benzene.

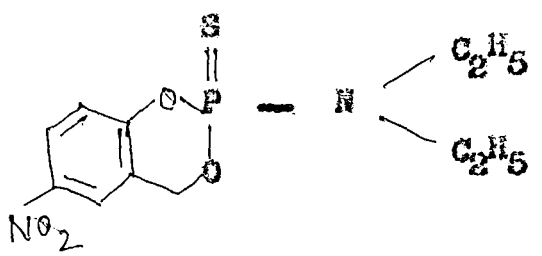
Analysis:

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

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

Molecular weight : 316.

6(b) (iii). 2- Diethylamido-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-Sulphide (BD-12):



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

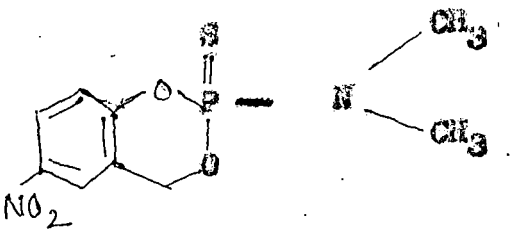
Analysis:

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

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

Molecular weight : 302.

6.(b)(iv). 2-Dimethylamido-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-Sulphide (BD-13):



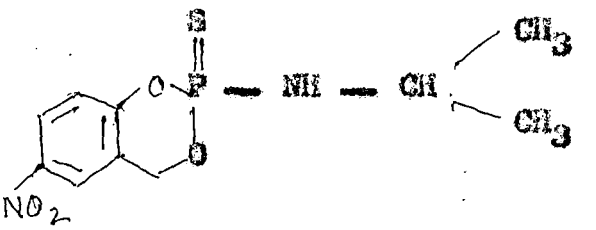
5-Nitro - Saligenin (1.69 gm, 0.01 mole), dimethylamido-phosphorodichloridothionate (1.78 gm, 0.01 mole), K_2CO_3 (2.76 gm, 0.02 mole) in 50 ml acetone gave BD-13 (2.0 gm, 75% yield) as white crystals (after working up as in BD-10), m.p. 128°C.

Analysis:

Found	: C, 39.40% ; H, 4.00% ; N, 10.19%;
Calc.for	: $C_9H_{11}O_4N_2PS$; C, 39.41% ; H, 4.01%; N, 10.21%.

Molecular weight : 274.

6.(b)(v). 2-Isopropylamido-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-Sulphide (BD-14):



5-Nitro-Saligenin (1.69 gm, 0.01 mole) isopropylamidophos-phorodichloridethionate (1.92 gm, 0.01 mole), K_2CO_3 (2.76 gm, 0.02

mole) in 50 ml acetone gave ED-14 (2.0 gm, 70% yield) as white crystals, m.p. 98°C.

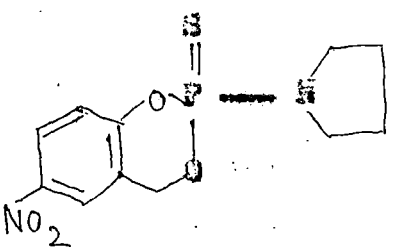
Analysis:

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

Calc. for : $C_{10}H_{13}O_4N_2PS$: C, 41.66% ; H, 4.61%; N, 9.72%.

Molecular weight : 288.

6.(b)(vi). 2-Pyrrolidino-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-Sulphide (ED-15):



5-Nitro - Saligenin (2.70 gm, 0.016 mole), pyrrolidinophosphorodichloridothionate (3.48 gm, 0.016 mole), K_2CO_3 (4.5 gm, 0.032 mole) in 50 ml acetone gave ED-15 (4 gm, 83% yield) as white crystals, m.p. 134°C.

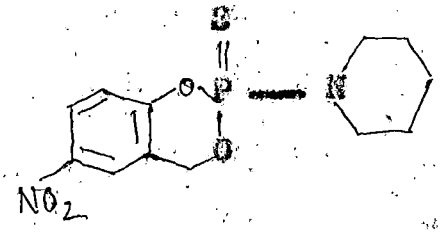
Analysis:

Found : C, 44.82% ; H, 4.27% ; N, 9.28%;

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

Molecular weight : 300.6.

6.(b)(vii). 2-Piperidino-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-Sulphide (BD-16):



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

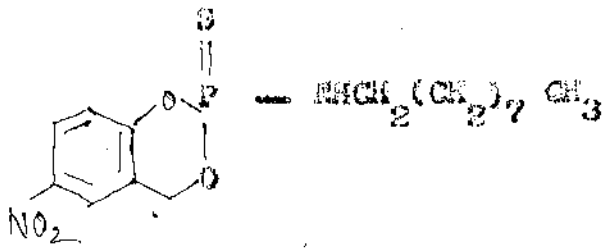
Analysis:

Found : C, 45.73% ; H, 4.71% ; N, 8.83%;

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

Molecular weight : 314.6

8.(b) (viii). 2-Nonylamido-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-sulphide (BD-17):



6-Nitro-saligenin (1.7 gm, 0.0103 mole) nonylamidophosphorodichloridothidonate (2.83 gm, 0.0103 mole), K_2CO_3 (2.9 gm, 0.0316 mole) in 50 ml acetone gave BD-17 (3.5 gm, 87% yield) as crystals, m.p. 64°C.

Analysis:

Found : C, 51.88% ; H, 6.69% ; N, 7.50%;

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

Molecular weight : 372.

7. ORAL INSECTICIDAL ACTIVITY ON COCKROACHES:

Insecticidal tests were performed on the American Cockroach, Periplaneta americana (Linn) according to Busvine with minor modifications[5]. Adults of P. americana weighing about 1 gm to 1.2 gm were collected in the month of May - June, 1980 from one particular location of the North Bengal University Campus. In the field they were never exposed to any organophosphorus insecticides. For preliminary experiment, ten roaches in each pot were exposed to different dosages of the compounds in dry sugar bait and after 48 hours the mortality was determined, and approximate 100% mortality (LC_{100}) value range (μg/gm basis) was found out.

To determine more precise LC_{100} value of each compound, one cockroach of known weight in each pot was exposed to known quantity of the alkylamidophosphorothionates progressively increasing its concentration by 1 μg for the dimethyl amido compound (range 15-20 μg/gm) and for the other compounds the concentration was increased by 2 μg/gm; for salithion and the methoxy compound (BD-8) the concentration was increased by 1 μg/gm. Each experiment was triplicated and the average LC_{100} value was found out by using the simple arithmetical procedure[6]. Before conducting the experiments on them, the cockroaches were kept starved for 24 hours. However, the varying susceptibility of male and female roaches to different compounds were ignored during the experiment.

8. ANTIFUNGAL ACTIVITY.

Helminthosporium Spp. was employed for the test of antifungal activity by using poisoned food technique[7]. Acetone solution of suitable quantity of the alkylamidophosphorothionates in sterile water containing 0.2 per cent Triton-X was incorporated into melted malt agar so as to get the concentration 5 - 100 ppm for the isopropylamido compound (BD-14) and 50 - 500 ppm for the other compounds in the media. The test medium was poured into sterile petridishes and after solidification the 7 mm 8 days old culture disc was placed asceptically at the centre of the petridish. Three replications on each test with appropriate control under same conditions were maintained. These petridishes were incubated at 26 ± 1°C for 48 hours and then the observations on the diameter of the colony were recorded. Percent inhibition over control was calculated following the equation given by Vincent[8].

9. ACCUTE ORAL TOXICITY TESTS ON RATS.

Oral toxicity testing was conducted on 6-12 months old male white albino rats, weighing 140-200 gm, each housed in separate compartments of a cage. All animals had free access to food and water. Different dosages of a compound were mixed with boiled fish and given to the animals at their habitual[9] feeding time. The mortality within 48 hours were recorded along with the toxic symptoms. Acute oral toxic dosage was found out by varying the amount of compound proportionately. The negligible amount

of compound wasted by the animal during dieting was roughly accounted for in determining the dosage.

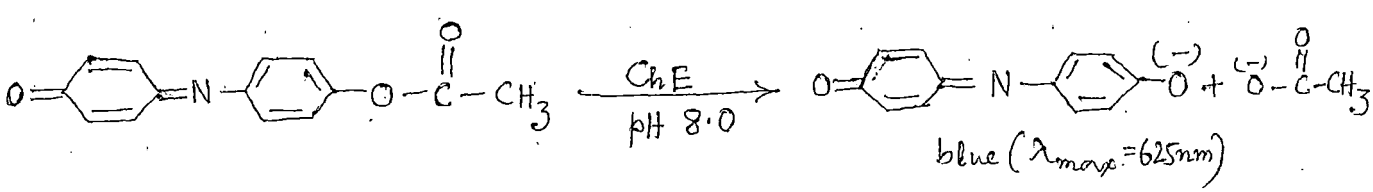
10. ANTICHOLINESTERASE ACTIVITY:

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

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

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

The acetyl cholinesterase inhibition of housefly head brie (HFAChE) has been measured by colorimetric method of Kramer and Gamson[10,11], using indophenyl acetate as an internal substrate - indicator in 0.05 M phosphate buffer of pH 8.0. The enzymatic reaction for indophenyl acetate is as follows:



The reaction mixture contained 5 ml buffer containing housefly head brei (1 ml of this solution contained $\frac{1}{4}$ fly-head) and 0.15 ml indophenyl acetate (total volume = 5.15 ml, Conc. of indophenyl acetate $\sim 10^{-5}$M). The readings for control and sample were taken at 625 nm after exactly 30 minutes incubation.

10.(a)(i) REAGENTS:

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

6.8 gm KH_2PO_4 dissolved in 500 ml of water was mixed with 475 ml of 0.1 N NaOH solution and diluted to 1 litre after the pH was adjusted to 8.0.

2. Indophenyl acetate : Working solution:

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

3. Glycerol solution:

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

4. Saline solution : 0.9%

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

5. Salt solution:

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

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

About 500 houseflies (Musca domestica) closed in a glass vessel were stored in a deep freeze for 1-2 hours. They were then transferred in a container with finely broken dry ice, removed individually from the container, decapitated with a shaving blade and forceps. 400 heads were combined with 2 ml of salt solution and 2 gms of washed sand in a prechilled size No. 1 mortar. The heads were slowly ground, then transferred to 50 ml plastic centrifuge tube with one 3 ml aliquots of cold saline solution and two 5 ml aliquots of buffer solution. The head fragments were removed by centrifugation for 10 minutes at 10,000 r.p.m. in superspeed centrifuge at 4°C. The supernatant liquid was decanted into a graduated cylinder and the fragmented heads were mixed with 10 ml of buffer solution and centrifuged again at 10,000 r.p.m. This extraction procedure was repeated twice. The supernatant solutions were combined and the volume was adjusted to 80 ml with the buffer solution so that each ml was equivalent to 5 fly-heads. This solution was stocked frozen in deep freeze. One ml of this solution was diluted to 5 ml with buffer solution so that each ml of the diluted solution contained single fly-head and used for each set of the

experiment.

10.(a)(ii) METHOD:

A series of 15 ml pyrex beakers (numbered 1,2,3 ....etc.) containing different amounts of inhibitor (viz., BD-10/BD-11/etc.) in acetone along with one marked 'control' without inhibitors were arranged. 0.5 ml of glycerol solution was poured in each beaker including the 'control'. The acetone (in beaker 1,2,3, etc.) was removed by blowing cold air.

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

After exactly 30 minutes, 0.15 ml of the indophenyl acetate solution (3.3×10^{-3} M) was added to the beaker marked 'control' and subsequently to each beaker of the series at the interval of 2 minutes and then kept to be incubated at 30°C. After incubation for exactly 30 minutes the aborobances of 'control' and remaining solutions were successively noted in the spectrophotometer (Carl-Zeiss Specol, *ZV*) at 625 nm with reference to enzyme-buffer (reagent blank) solution.

Calculation:

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

11. ANTICHOLINESTERASE ACTIVITY IN GOAT-WHOLE BLOOD:

The method employed to determine the inhibition of the activity of acetyl cholinesterase in goat whole blood by organophosphorus compounds was by colorimetric method of Kramer and Gamson [10,11], 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 (~~total volume = 5.15 ml, concentration of indophenyl acetate~~ in the reaction mixture $\sim 10^{-5}$ M). The readings of 'control' and 'sample' were taken at 625 nm, after exactly 30 minutes incubation.

11. (a) MATERIALS AND METHOD:

(i) Material:

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

The other reagents e.g. (b) indophenyl acetate (c) phosphate buffer solution and (d) glycerol solution were prepared just as the same as mentioned in 10(a).

(11) Method:

The Anticholinesterase Activity in Goat whole blood was determined by the same method as described in 10(a). The only exception was that the contents of the beaker was filtered through a 4.25 cm Whatman No. 1 filter paper after exactly 29 minutes incubation and the absorbance of the filtrate was noted in spectrophotometer at 625 nm with reference to the filtrate of the enzyme-buffer solution and % inhibition was calculated in the same way as described in 10(a).

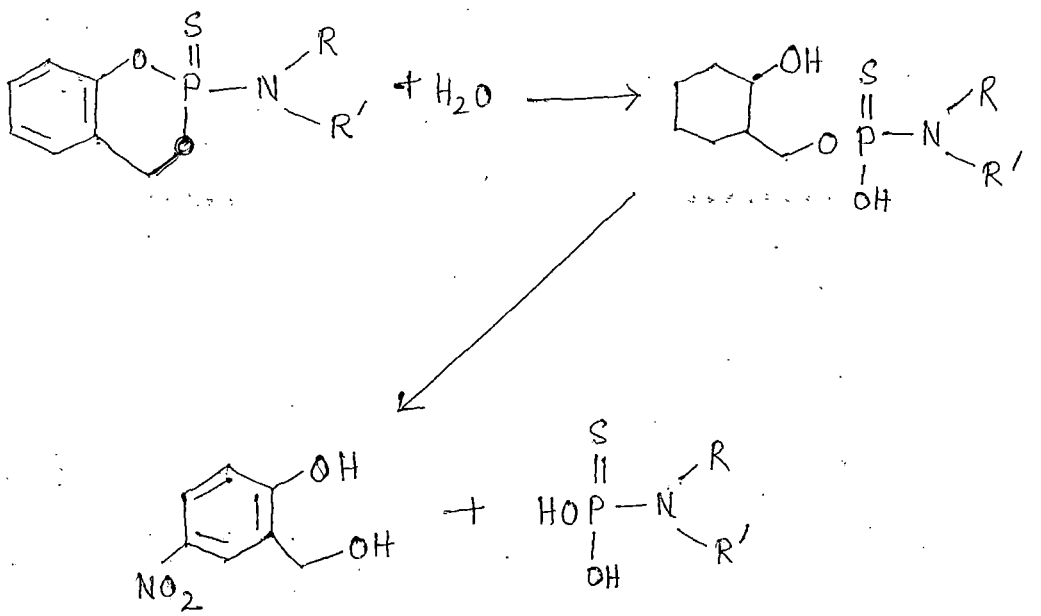
12. PHYTOTOXICITY TEST:

Phytotoxicity testing was conducted according to Eto et al (12). 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 500, 250 or 100 ppm of the compounds was poured into a petridish bottom covered with absorbent cotton. Ten seeds of wheat (Triticum. Sp. UP 262 variety supplied by National Seed Corporation of India) were planed 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.

13. CHEMICAL HYDROLYSIS:

Since the nitro saligenin cyclic alkylamidophosphorothionates are analogous to salithion (13), it is envisaged to proceed

with the initial fission of aryl ester bond in dioxaphosphorin ring followed by the liberation of nitro saligenin.



Moreover, the reactivity of nitro saligenin cyclic phosphorothionates is not only effected by exocyclic substituents on the phosphorus atom but also it is significantly influenced by the presence of nitro group at the para position to phenolic -OH group in the benzene ring.

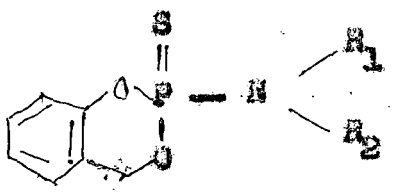
The chemical hydrolysis studies were performed in 0.0095 M NaOH solution in 50 per cent ethanol of pH 11.85 at 20°C. One ml of ethanol solution of the compounds was added with 9 ml of 0.0095 M NaOH solution (total volume 10 ml). The rate of hydrolysis was monitored by following the formation of nitro-saligenin anion at 400, 410, 420 and 430 nm in a Carl Zeiss Speckol UV spectrophotometer. UV spectra of the alk/lamidophosphorothionates and hydrolytic products were examined prior to Kinetic studies to show that overlap in relevant absorption peaks were not present. The concentration of the hydrolytic product was determined from the extinction co-efficient value of 5-nitro saligenin in the same 0.0095 M NaOH solution at the said wave lengths. The pseudo first order rate constants (K_{hyd}) were determined by the least squere regression analysis.

B. RESULTS AND DISCUSSION

B. RESULTS AND DISCUSSION:

1. SYNTHESIS.

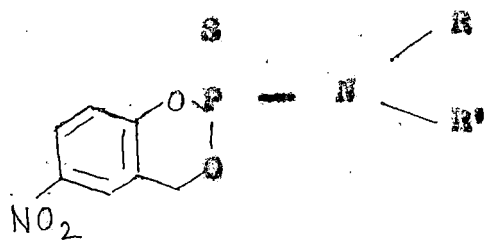
Only four saligenin cyclic phosphoramidothionates have so far been reported[14]. The physical properties and per cent yield of these compounds are given below.



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

All of them are liquids, and per cent yields are low (13-27%)[14]. The methylamido compound (K-35) has been prepared at -4°C using pyridine as dehydrogen - chloride agent in chloroform solution; the other three compounds (K-37, K-36, K-38) have been prepared at elevated temperatures (60-80°C) using anhydrous K_2CO_3 as dehydrogen -chloride agent and copper powder as catalyst in toluene with low yield.

We, however, succeeded to prepare the nitro-saligenin cyclic phosphoramidothionates (BD-10 to BD-17) in solid crystalline form with high yields (Table, given below):



Code No.	Amido Group	Yield (%)	m.p. (°C)
BD - 10	Cyclohexylamido	70 - 90	125
BD - 11	Morpholino	60 - 80	149
BD - 12	Diethylamido	80 - 90	105
BD - 13	Dimethylamido	75 - 80	128
BD - 14	Isopropylamido	70 - 80	98
BD - 15	Pyrrolidino	80 - 90	134
BD - 16	Piperidino	80 - 90	130
BD - 17	Nonylamido	80 - 90	64

The method[14] due to Eto *et al* by employing K_2CO_3 and copper powder at elevated temperature in benzene was tried for the preparation of the dimethylamido compound (BD-13); in that case, a pasty material was obtained from which it was difficult to get the pure compound.

Attempts had also been made several times to prepare the 2-methylamido-6-nitro-4H-1,3,2-benzodioxnphosphorin-2-sulphide, and

its ethylamido analog; unfortunately, in each case a viscous yellowish liquid was obtained. TLC of these liquids gave several spots indicating the presence of a number of compounds; the pure amidophosphorothionates could not be isolated from these liquid mixtures.

Cremlyn and Akhtar[15] had reported that the morpholinophosphorodichloridothionate is a liquid (b.p. 100-102/1.5 mm); Fusco and Bertulli[16] has also reported the same (b.p. 107-110/2 mm). We could, however, succeed to obtain the morpholinophosphorodichloridothionate in the form of white crystalline solid (m.p. 30-31°C).

2. SPECTRAL PROPERTIES:

The structures of the alkylamidophosphorothionates have been determined by chemical analysis and UV, IR, mass and NMR spectra. The analytical data along with the physical characteristics have been presented previously (Section A).

The spectral data for different alkylamidophosphorothionates are given below:

3. SPECTRAL DATA:

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

UV (Fig. 4): λ_{max}^{EtOH} = 290 nm (log ϵ = 4.051)

IR (Fig. 5):

1020 Cm^{-1} (s), P-O-C (alkyl);

1240 Cm^{-1} (vs) and 880-920 Cm^{-1} (s), P-O-C (aryl);

1515 Cm^{-1} (s), asym. str. of nitro group;

1340 Cm^{-1} (s), sym. str. of nitro group;

800 Cm^{-1} (s), P = S (I);

650 Cm^{-1} (m), P = S (II);

3300 Cm^{-1} (m), N - H str.;

1620 Cm^{-1} (w) and 1585 Cm^{-1} (m), two components of the substituted benzene ring "quadrant stretching" C = C vibrations;

1480 Cm^{-1} (m) and 1420 Cm^{-1} (m), two components of the substituted benzene ring "semicircle stretching" C = C vibrations;

740 Cm^{-1} (m), P - N str. (also aromatic C-H-O bending).

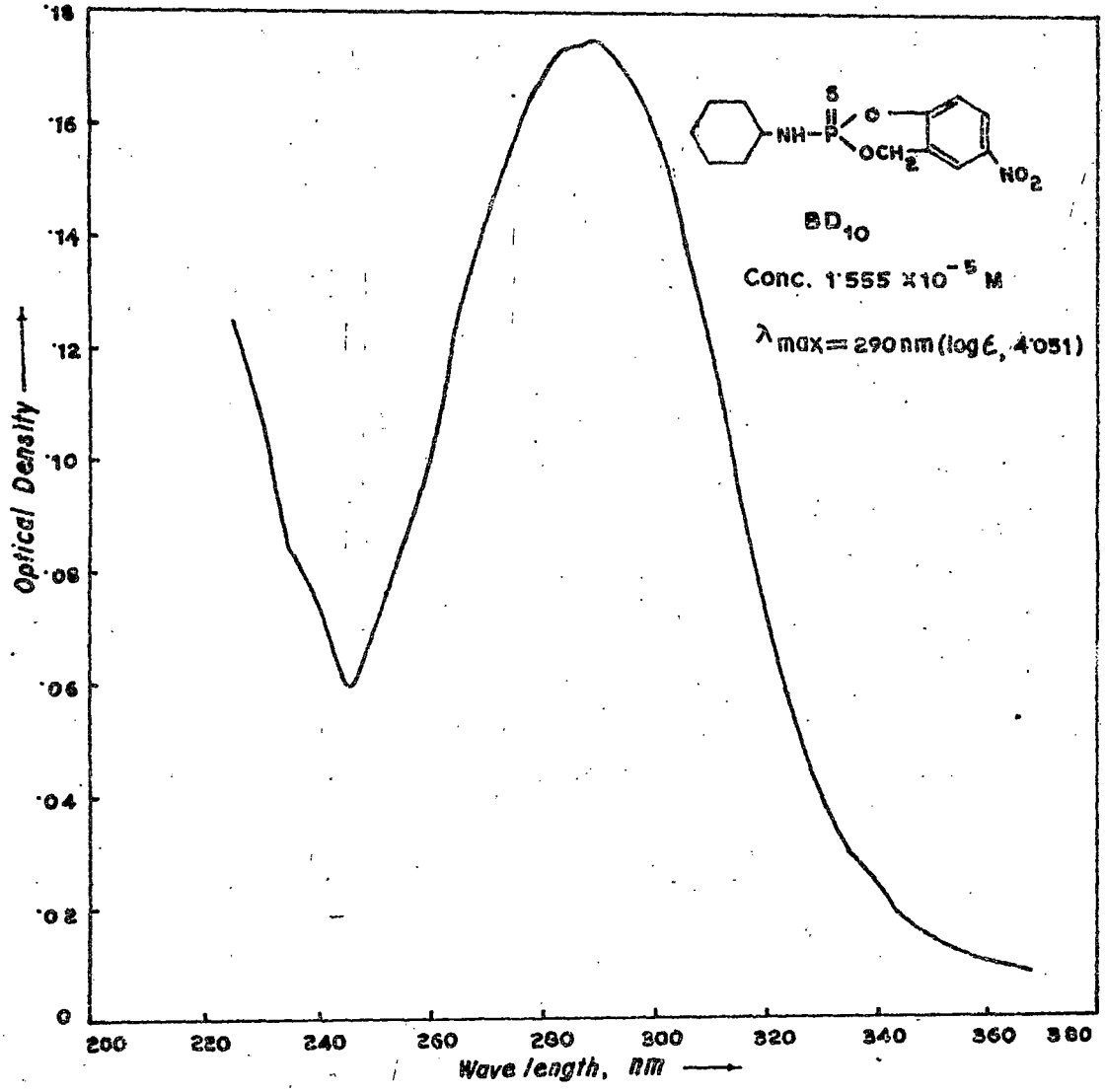


Fig. 4. UV spectrum of BD_{10} in ethanol

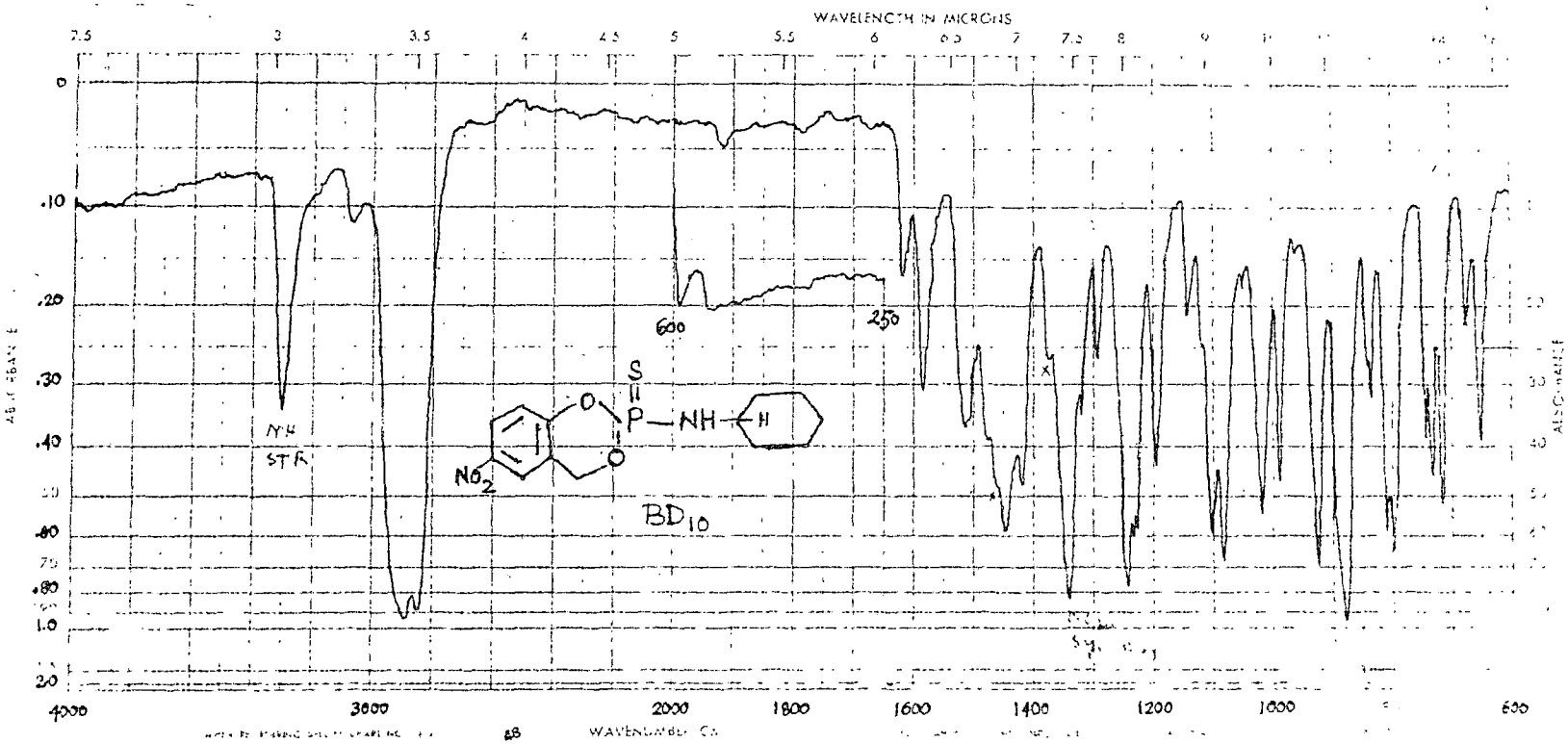


Fig. 5. IR spectrum of 2-Cyclohexylamido-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-sulphide.

Mass (Fig. 6):

m/e	%RI
328 (M^+)	34.5
296	43.5
295 (base peak)	100
279	45.0
249	33.0
230	35.0
192	54.5
162	69.0

^1H NMR δ ($CDCl_3$) ppm (Fig. 7):

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

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

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

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

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

5.45 (2H, Octet, $-CH_2-$ group in dioxaphosphorin ring);

7.1 (1H, doublet, J = 8.5 Hz, one aromatic hydrogen meta to nitro group);

8.05 (1H, doublet, one aromatic hydrogen ortho to both nitro group and $-CH_2-$ group of dioxaphosphorin ring);

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

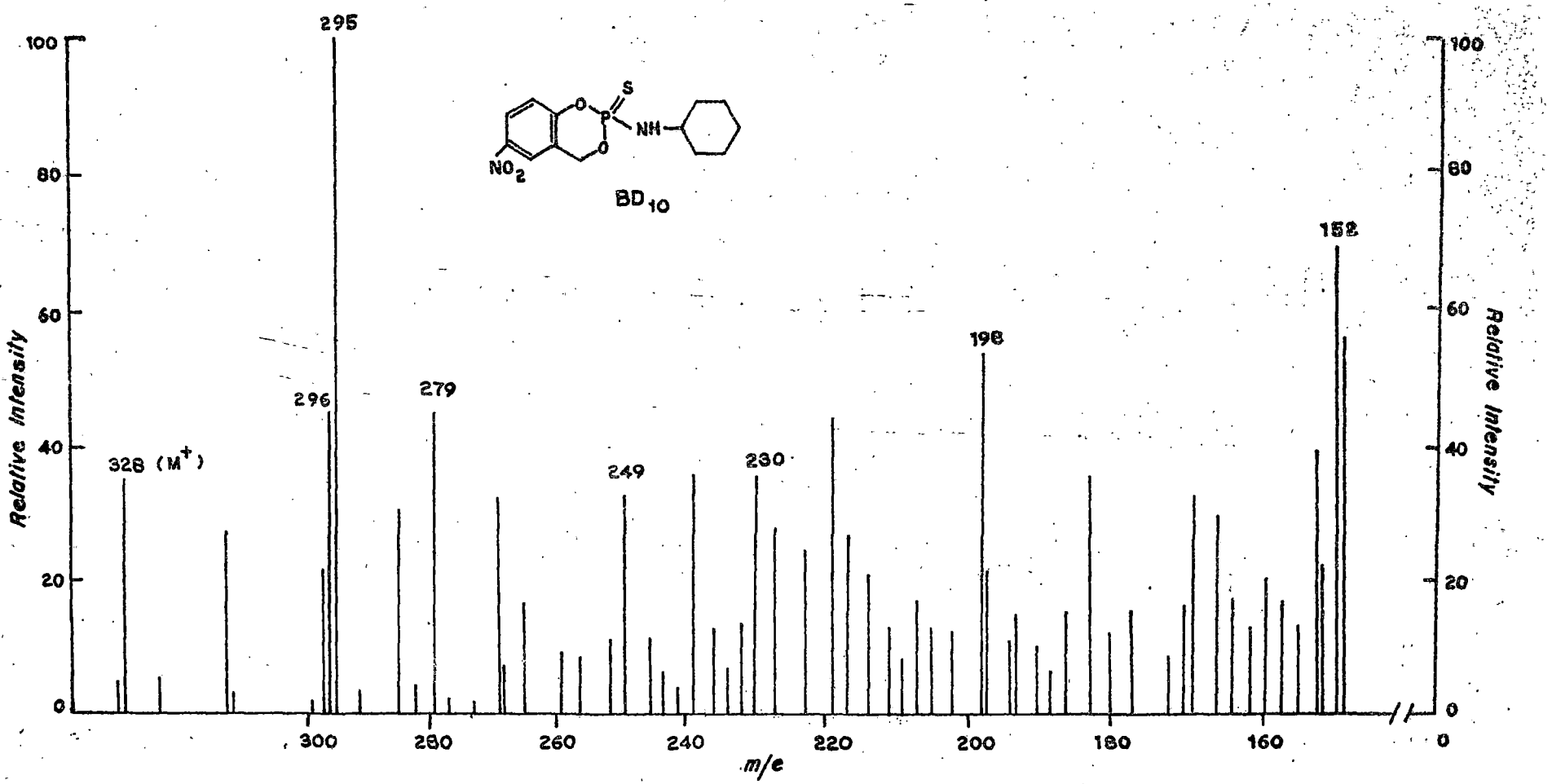


Fig. 6. Mass spectra of -2- Cyclohexylamido - 6- nitro 4H- 1,3.2 benzodioxaphosphorin-2- sulphide.

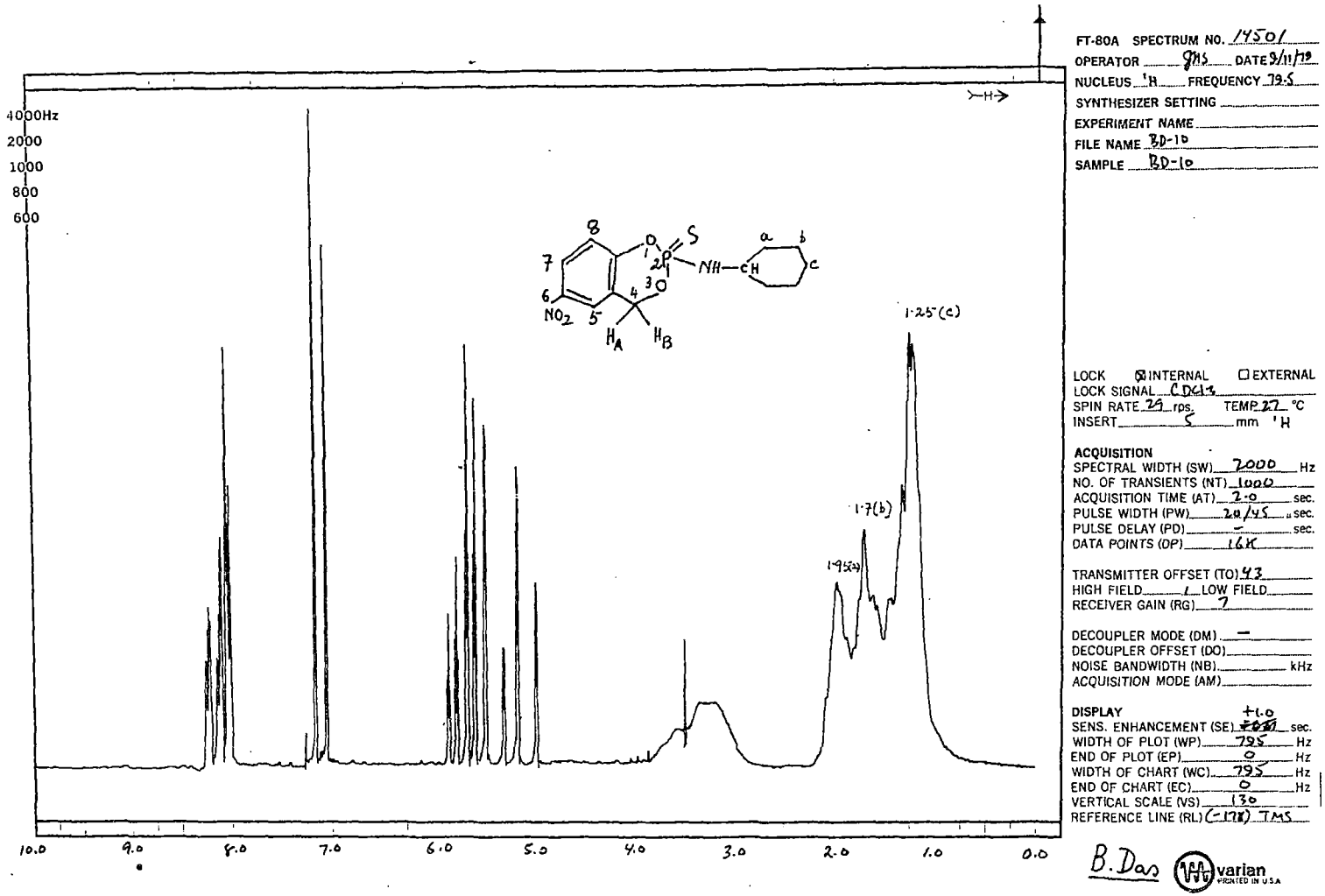


Fig. 7. ^1H NMR spectrum of 2-Cyclohexylamido-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-sulphide.

(11) 2-Morpholino-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-sulphide (ED-11):

UV (Fig. 8): λ_{max}^{EtOH} = 274 nm (log ϵ = 4.908)

IR (Fig.9):

1020 Cm^{-1} (s) and 980 Cm^{-1} (vs), P-O-C (alkyl);
1250 Cm^{-1} (vs) and 886 Cm^{-1} (s), P-O-C (aryl);
1520 Cm^{-1} (s), asym. str. of nitro group;
1340 Cm^{-1} (vs), sym. str. of nitro group;
820 Cm^{-1} (s), P = S (I);
650 Cm^{-1} (m), P = S (II);
720 Cm^{-1} (m), P - N str. ;
1620 Cm^{-1} (w) and 1585 Cm^{-1} (m), benzene ring "quadrent stretching" vibrations.

Mass (Fig. 10):

m/e	%RI
316 (M^+)	43.0
284	60.0
283 (base peak)	100
230	48.6
198	70.0
152	46.4

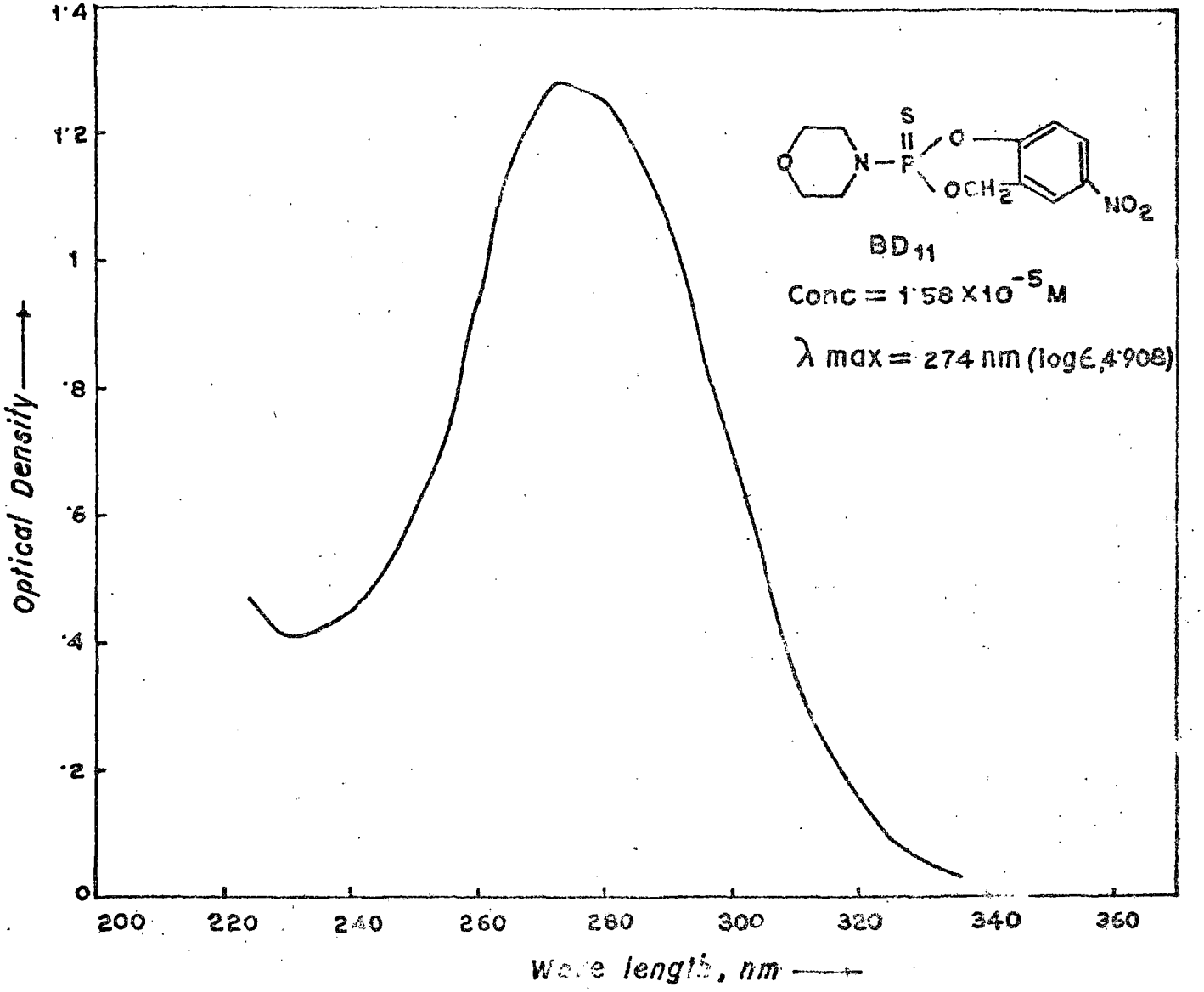


Fig. 8 UV spectrum of BD_{11} in ethanol

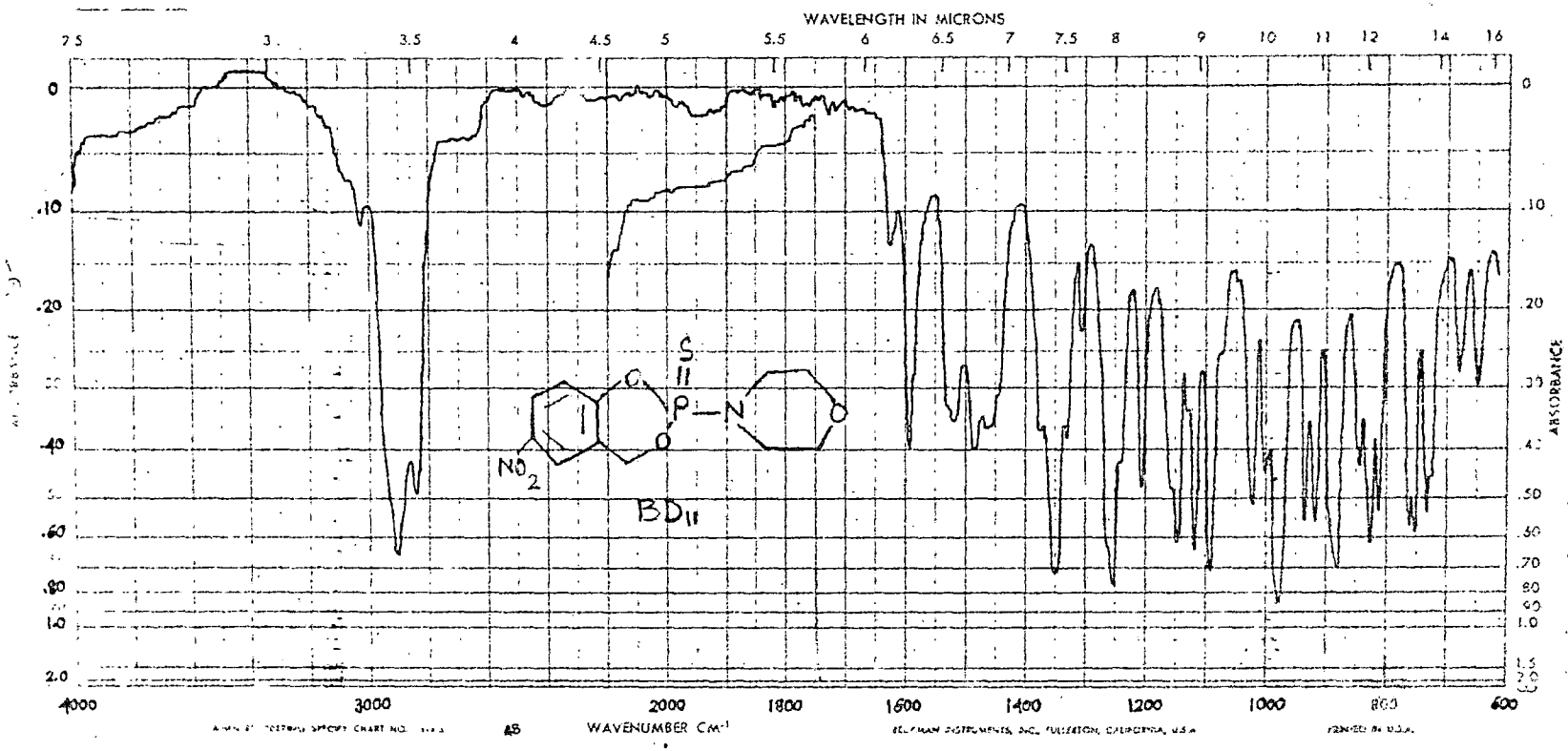


Fig. 9. IR spectrum of 2-Morpholino-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-sulphide

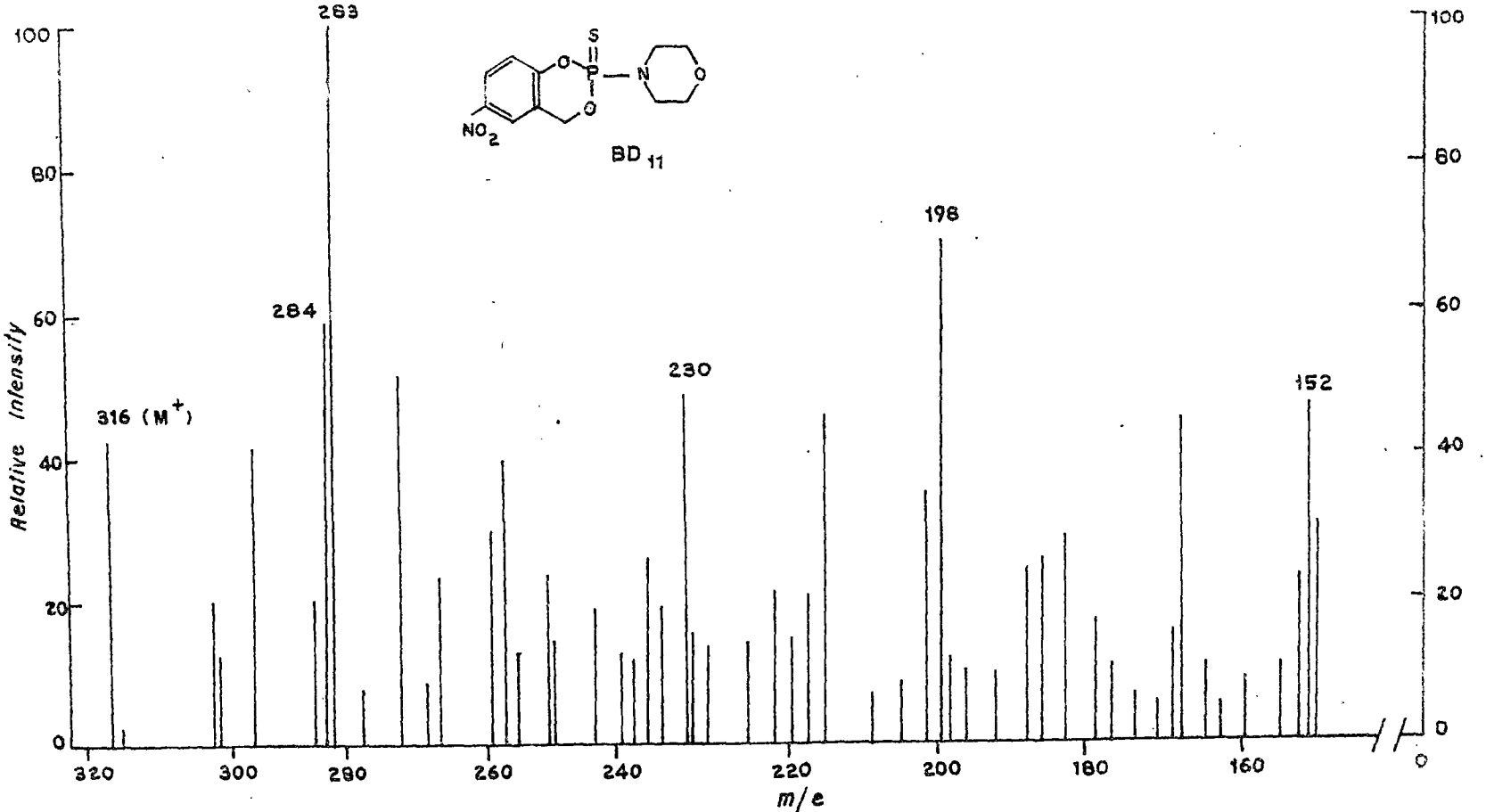


Fig. 10. Mass spectra of 2- Morpholino-6-nitro 4H-1,3,2 benzodioxaphosphorin-2 sulphide.

^{1}H NMR δ($CDCl_3$) ppm (Fig. 11):

3.6 (4H, multiplet, $-N(-CH_2-)(-CH_2-)$ group of the morpholine ring);

3.8 (4H, multiplet, $O(-CH_2-)(-CH_2-)$ group of the morpholine ring);

5.45 (2H, Octet, $-CH_2-$ group in dioxaphosphorin ring);

7.1 (1H, doublet, one aromatic hydrogen meta to nitro group);

8.05 (1H, doublet, one aromatic hydrogen ortho to both nitro group and $-CH_2-$ group of dioxaphosphorin ring);

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

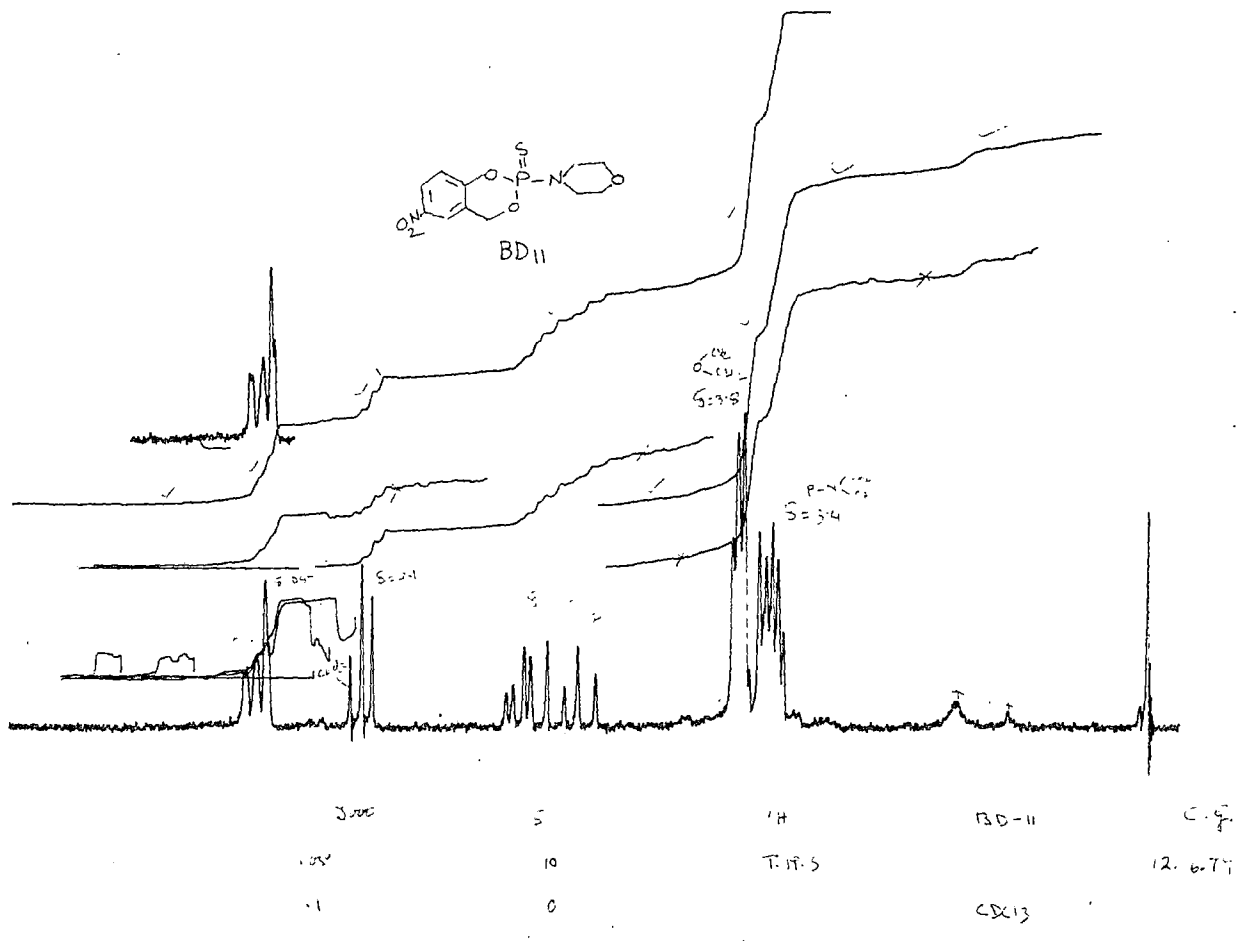


Fig. 11: 1

(iii) 2-N,N - Diethylamido-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-sulphide (BD-12):

UV (Fig. 12): λ_{max}^{EtOH} = 295 nm (log ϵ =3.979)

IR (Fig.13):

1030 Cm^{-1} (vs), P-O-C (alkyl);
1235 Cm^{-1} (vs) and 880 Cm^{-1} (vs), P-O-C (aryl);
1515 Cm^{-1} (s), asym. str. of nitro group;
1345 Cm^{-1} (s), sym. str. of nitro group;
890 Cm^{-1} (s), P = S (I);
640 Cm^{-1} (m), P = S (II);
786 Cm^{-1} (m), P - N str.;
1620 Cm^{-1} (w) and 1585 Cm^{-1} (s), benzene ring "quadrant stretching" vibration.

Mass (Fig. 14):

m/e	%RI
302 (M^+)	25
269 (base peak)	100
198	40
152	10

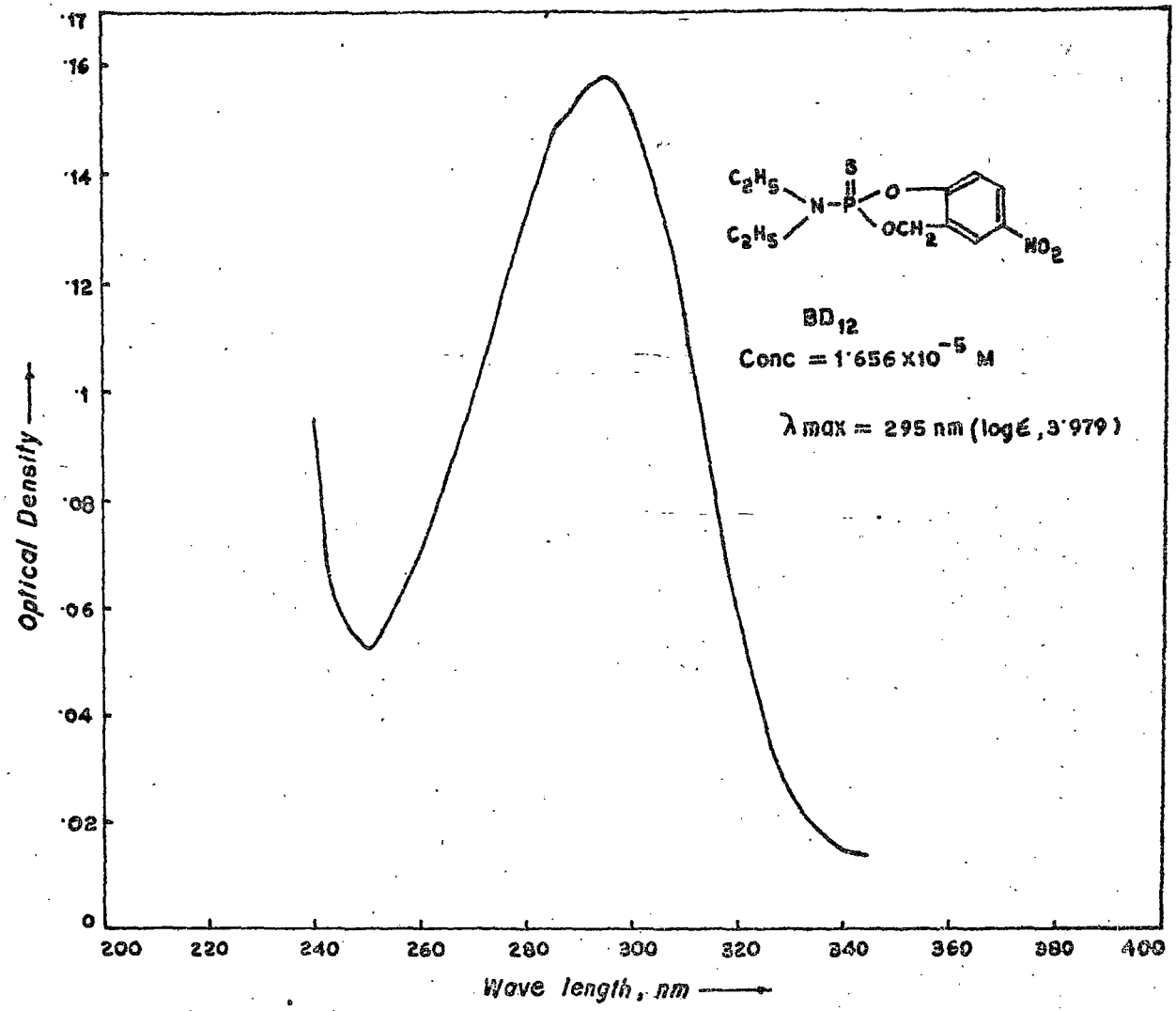


Fig. 12. UV spectrum of BD_{12} in ethanol

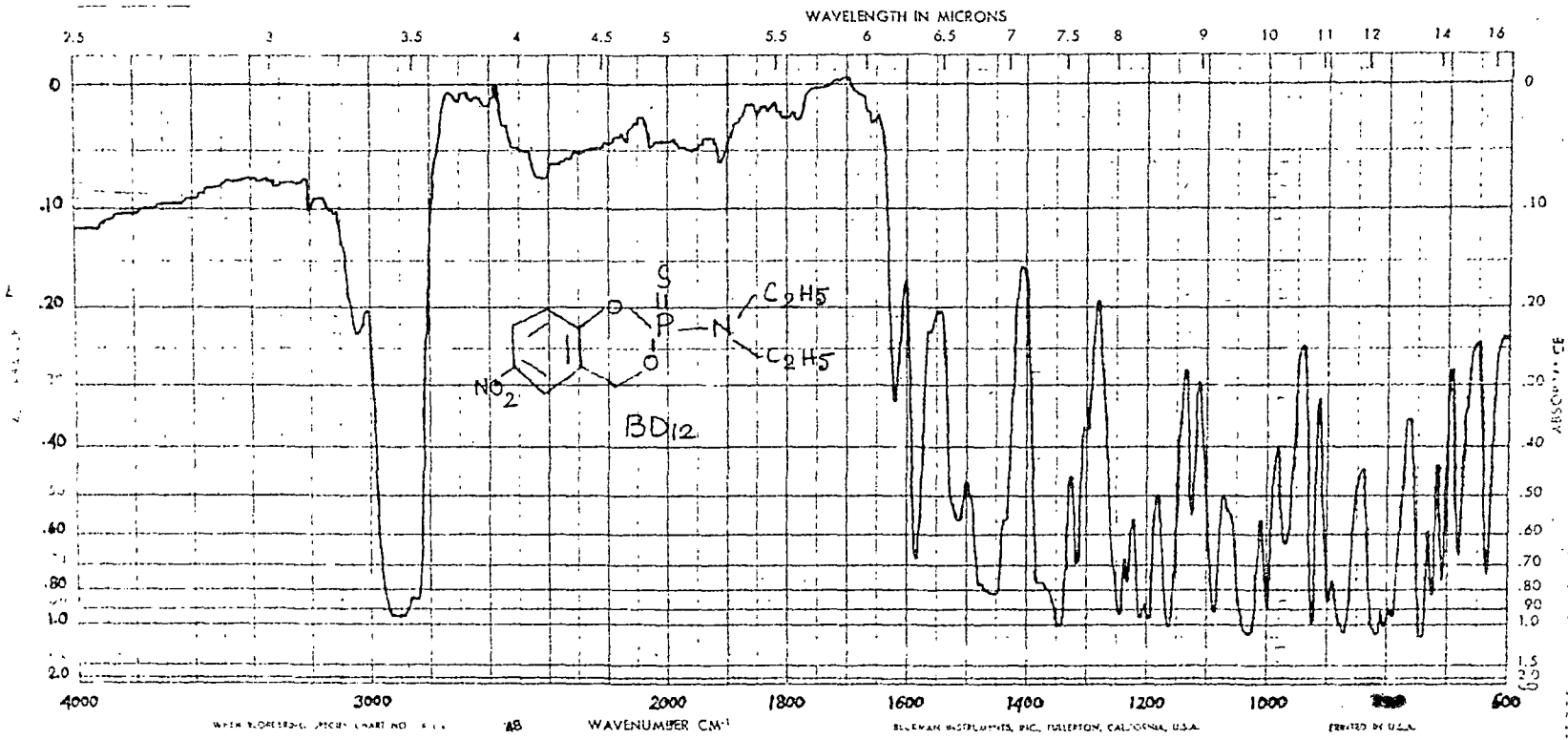


Fig. 13. IR spectrum of 2-N,N-Diethylamido-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-sulphide.

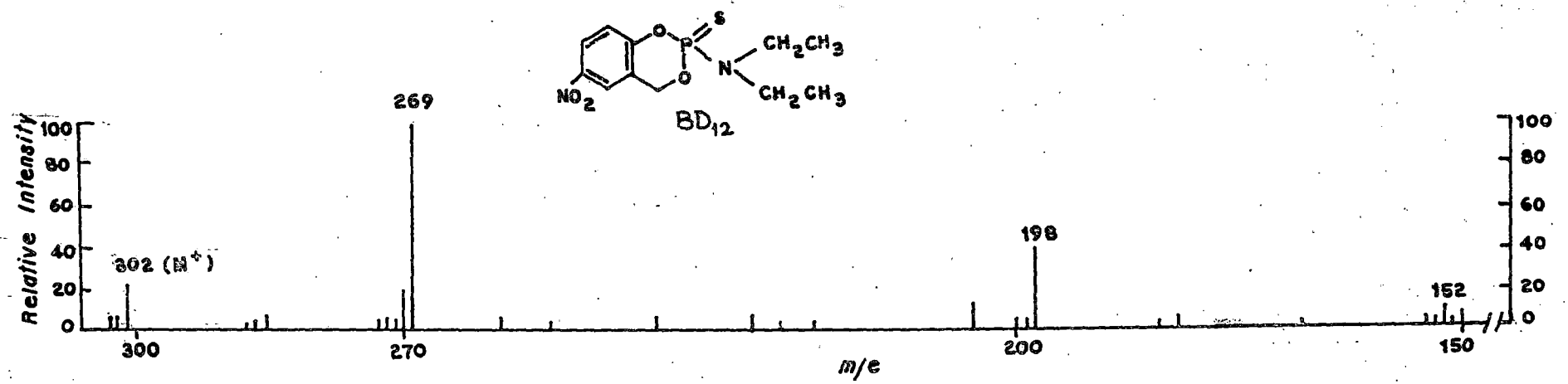


Fig. 14. Mass spectra of 2-diethyl amido-6-nitro 4H-1,3,2 benzodioxaphosphorin-2-sulphide.

^{1}H NMR δ ($CDCl_3$) ppm (Fig. 15):

1.15 (6H, triplet, J = 7.0, two $-CH_3$ groups);

3.4 (4H, multiplet, $-N\begin{matrix}\diagup CH_2- \\ \diagdown CH_2-\end{matrix}$ group);

5.4 (2H, multiplet, $-CH_2-$ group in the dioxaphosphorin ring);

7.05 (1H, doublet, one aromatic hydrogen meta to nitro group);

8.0 (1H, doublet, one aromatic hydrogen ortho to both nitro group and $-CH_2-$ group of dioxaphosphorin ring);

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

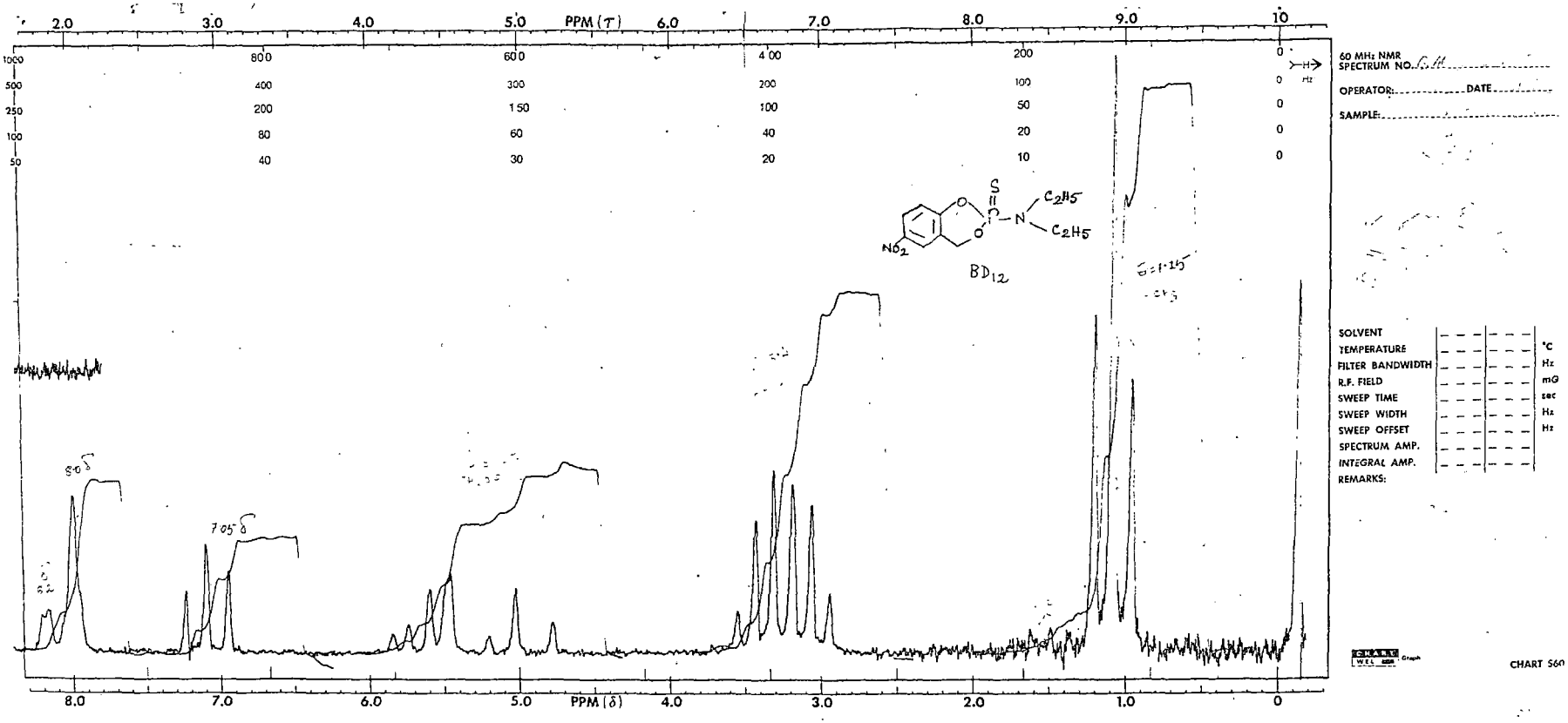


Fig. 15 - ^1H NMR of 2-N,N-Diethylamido-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-sulphide

(iv) 2 - N, N- Dimethylamido-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-sulphide (BD-13):

EtOH

UV (Fig. 16): λ_{max} = 295 nm (log ϵ =4.006)

IR (Fig. 17):

1010 Cm^{-1} (vs), P-O-C (alkyl);

1260 Cm^{-1} and 880 Cm^{-1} (s), P-O-C_6 (aryl);

1620 Cm^{-1} (s), asym. str. of nitro group;

1340 Cm^{-1} (s), sym. str. of nitro group;

820 Cm^{-1} (s), P = S (I);

645 Cm^{-1} (m), P = S (II);

780 Cm^{-1} (m), P - N str;

1620 Cm^{-1} and 1585 Cm^{-1}(s), benzene ring "quadrant stretching" vibration.

Mass (Fig. 18):

m/e	%RI
274 (M^+)	94.4
241	91.6
230	7.0
198 (base peak)	100
167	7.0
152	9.8

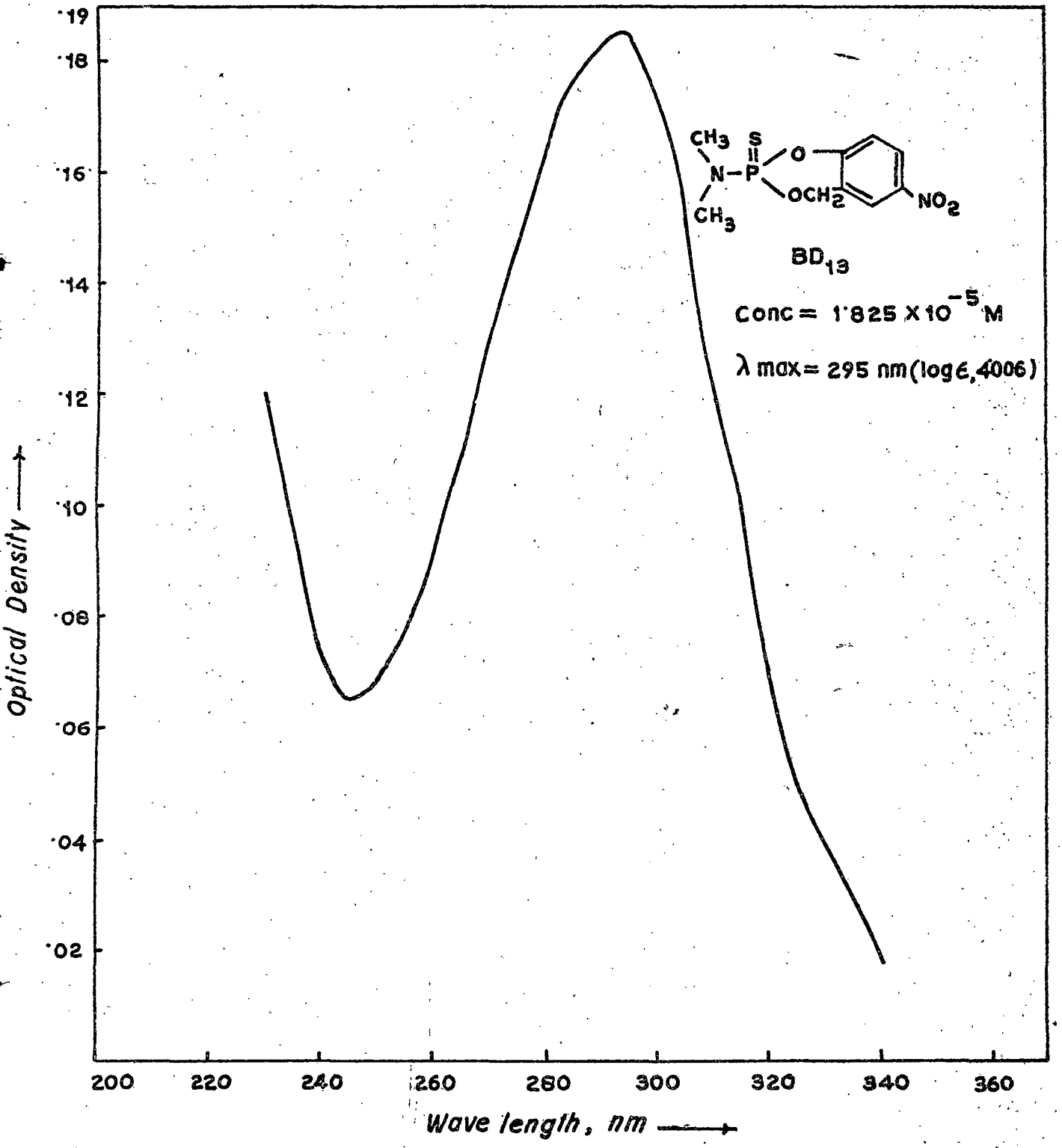


Fig. 16. UV spectrum of BD_{13} in ethanol.

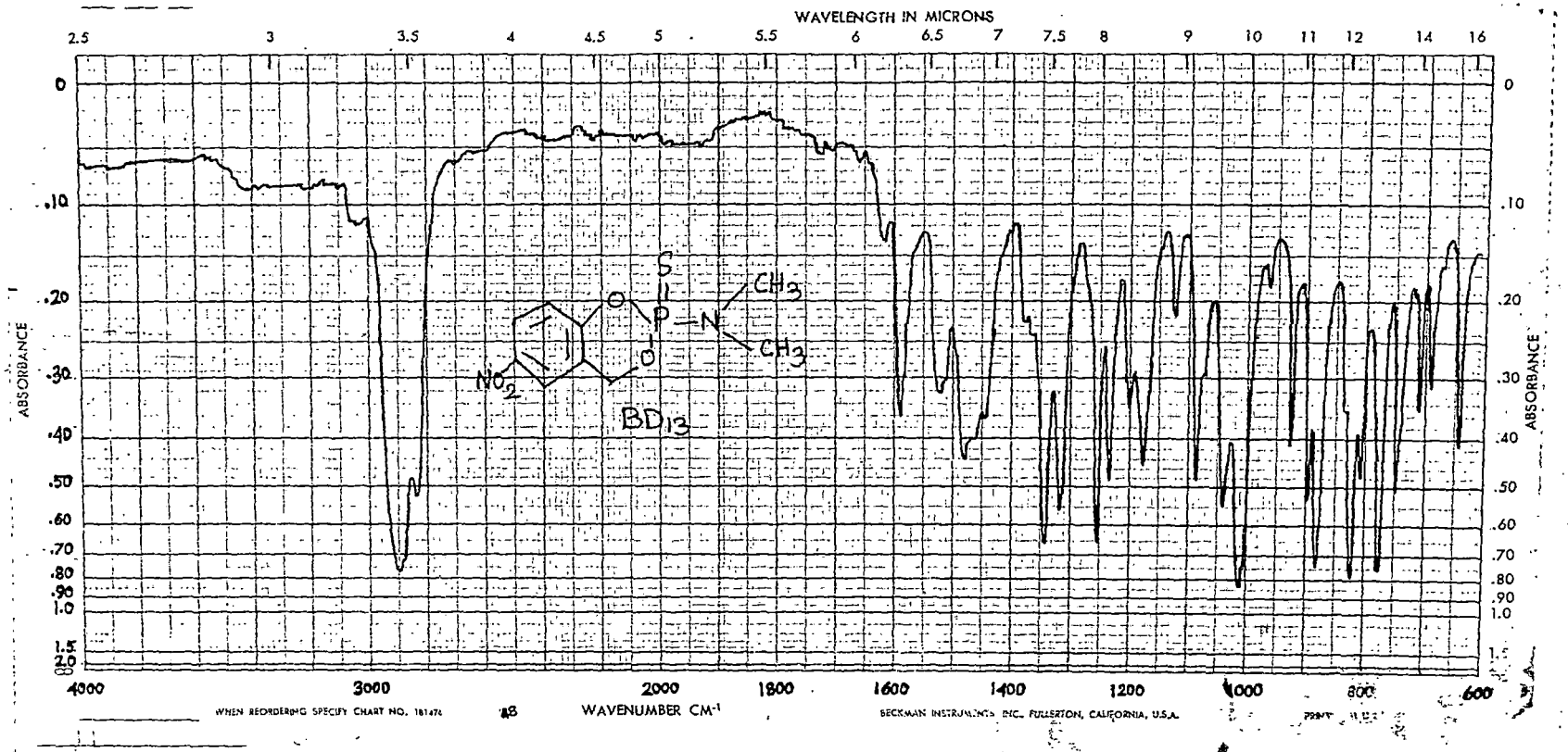


Fig. 17. IR spectrum of ~~2-~~ N,N-Diethylamido-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-sulphide

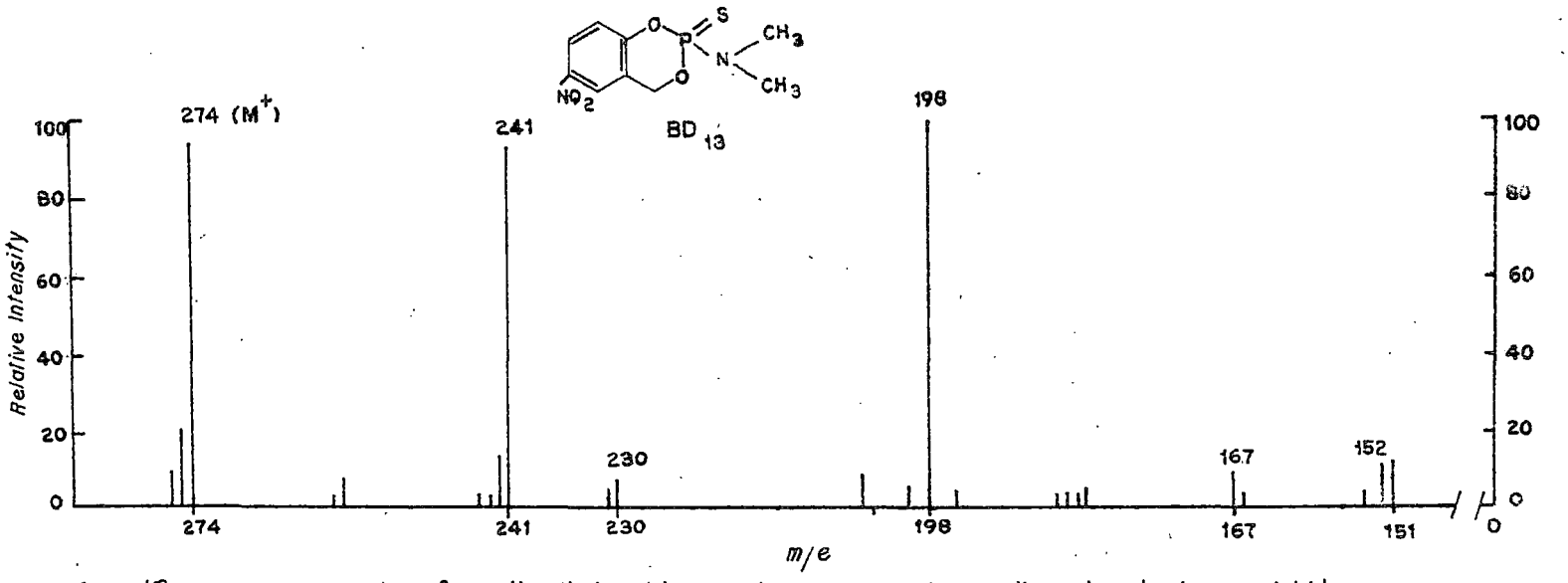


Fig. 18 Mass spectra of 2-dimethyl amido-6-nitro 4H-1,3,2 benzodioxaphosphorin-2 sulphide.

^1H NMR δ ($CDCl_3$) ppm (Fig. 19):

2.8 (6H, doublet, two $-CH_3$ groups in $-N(CH_3)_2$);

5.4 (2H, multiplet, $-CH_2-$ group in the dioxaphosphorin ring);

7.0 (1H, doublet, one aromatic hydrogen meta to nitro group);

8.0 (1H, one aromatic hydrogen ortho to both nitro group and $-CH_2-$ group of dioxaphosphorin ring);

8.18 (1H, multiplet, remaining one aromatic hydrogen).

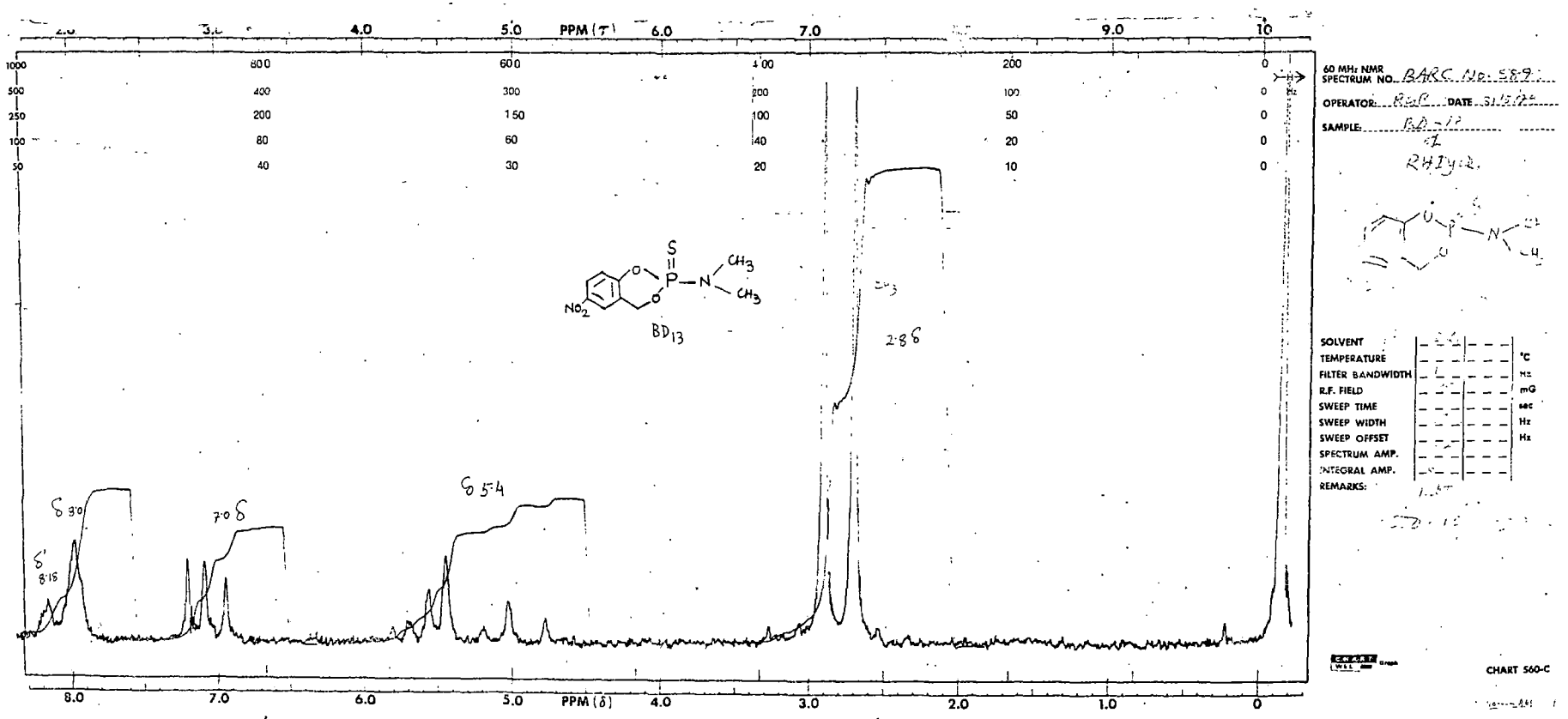


Fig. 19 – ^1H NMR spectrum of 2-N,N-Dimethylamido-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-sulphide

(v) 2-Isopropylamido-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-sulphide (BD-14):

EtOH

UV (Fig. 20): λ_{max} = 295 nm (log ϵ =3.915)

IR (Fig. 21):

1020 Cm^{-1} (vs), P-O-C (alkyl);

1240 Cm^{-1} (s) and 880 Cm^{-1} (s), P-O-C (aryl);

1515 Cm^{-1} (s), asym. str. of nitro group;

1340 Cm^{-1} (vs), sym. str. of nitro group;

800 Cm^{-1} (s), P = S (I);

660 Cm^{-1} (m), P = S (II);

1620 Cm^{-1} (w) and 1585 Cm^{-1} (s), benzene ring "quadrant stretching" vibration;

3290 Cm^{-1} (s), N - H str.

Mass (Fig. 22):

m/e	%RI
288 (M^+)	50
273	25
266	52
198 (base peak)	100
152	64

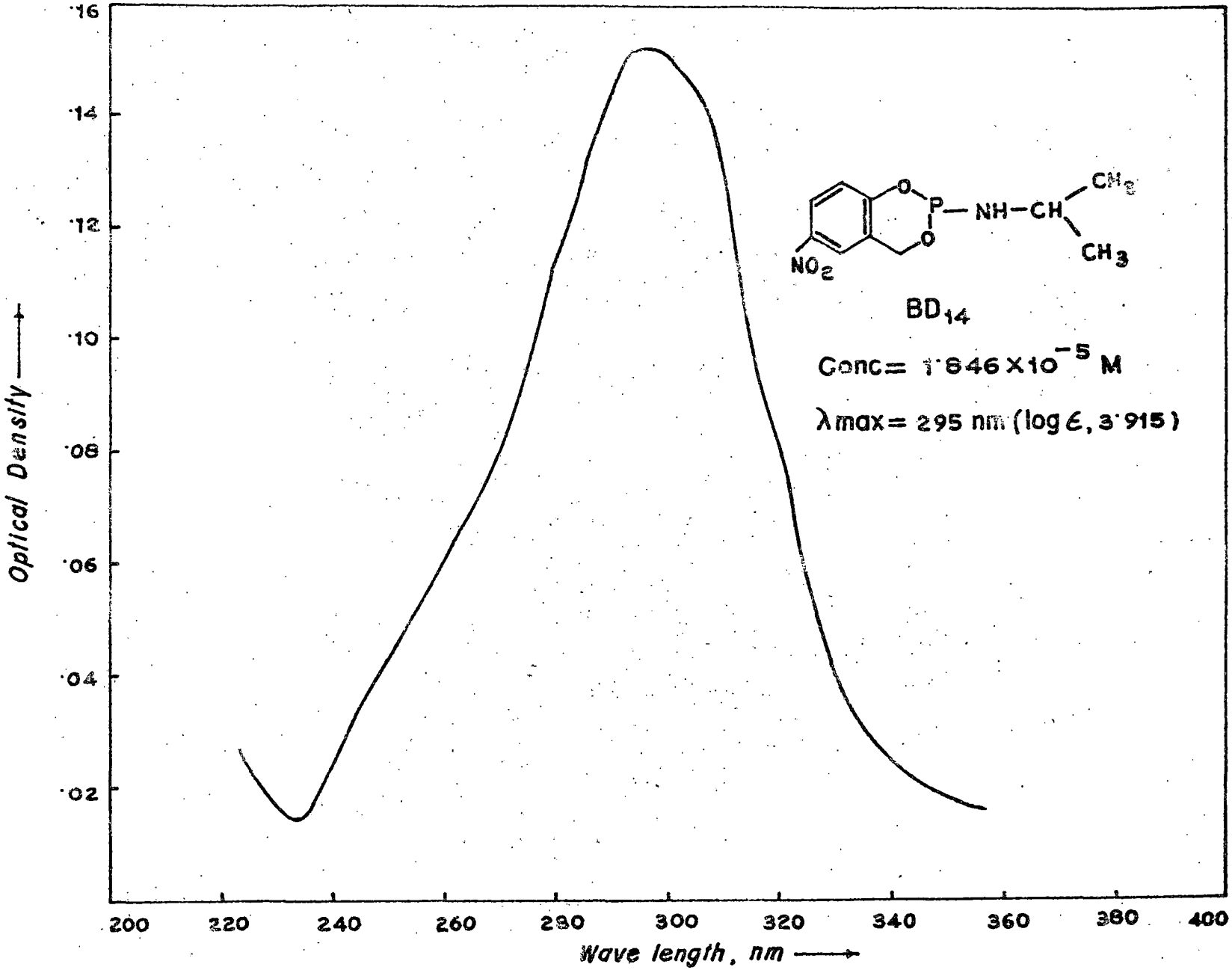


Fig. 20 UV spectrum of BD_{14} in ethanol

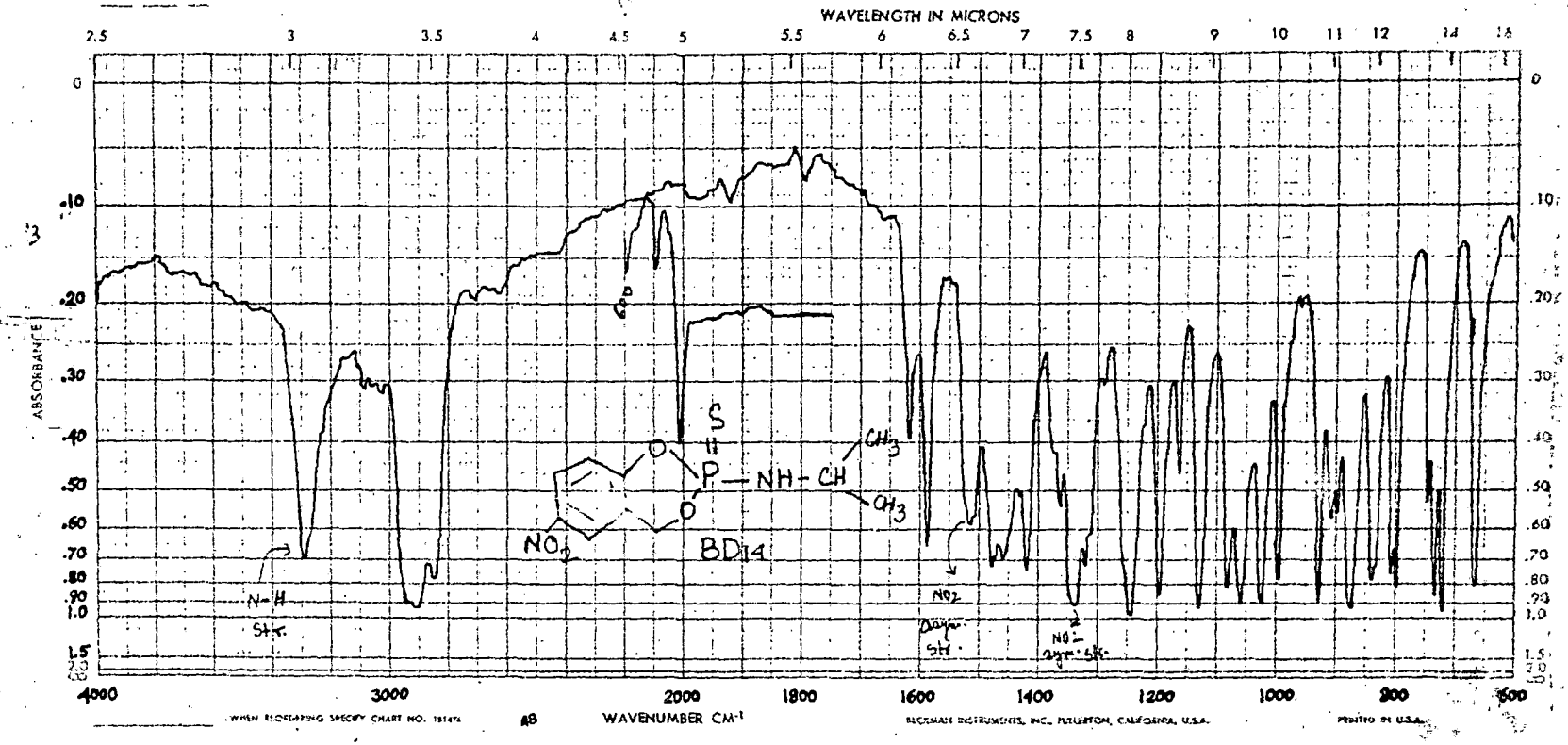


Fig. 21. IR spectrum of 2-Isopropylamido-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-sulphide

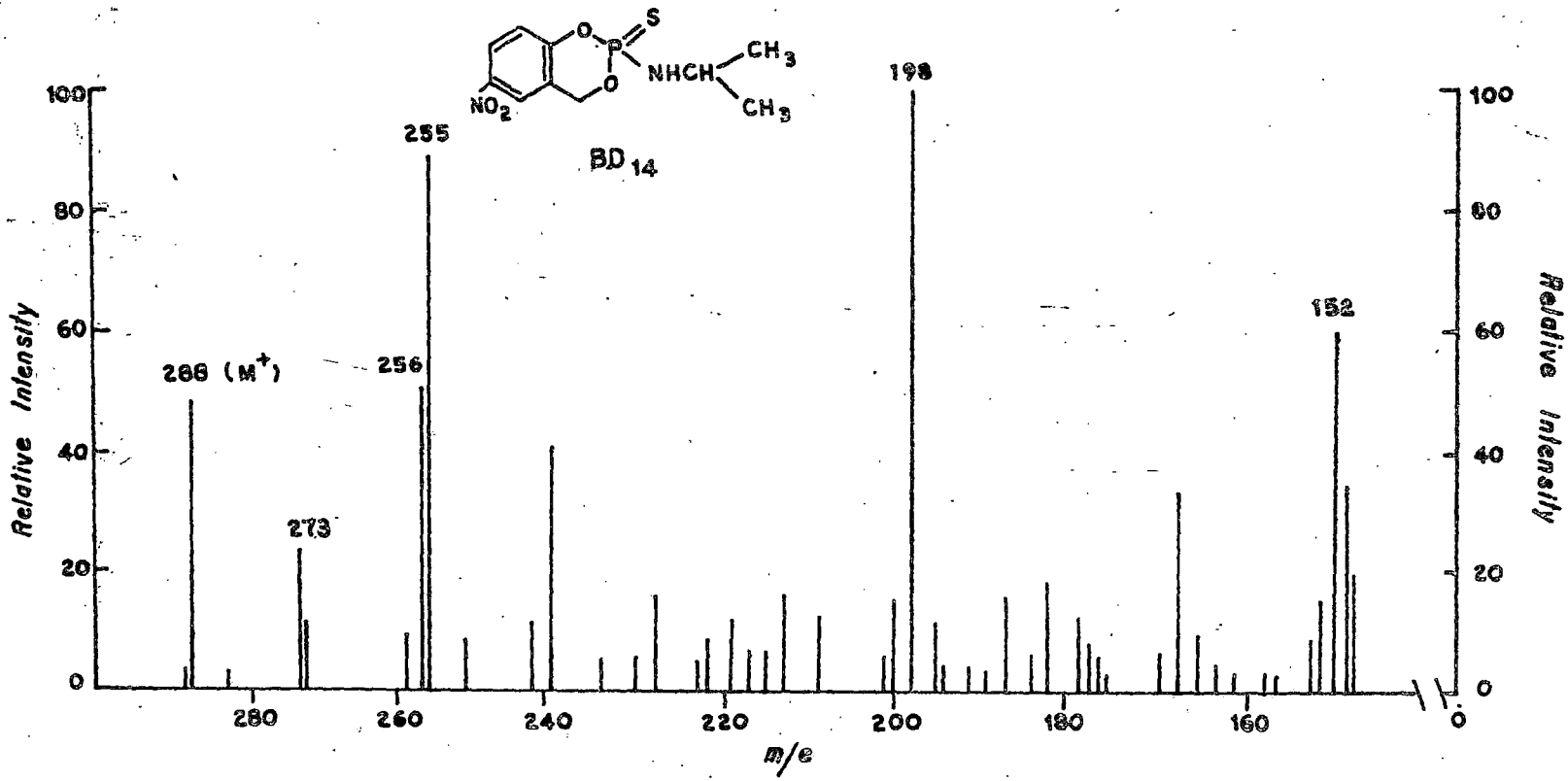


Fig. 22 Mass spectra of-2- Isopropylamido-6-nitro-4H-1,3,2 benzodioxaphophorin-2-sulphide.

^{1}H NMR $\delta(CDCl_3)$ ppm (Fig. 23):

1.25 (6H, doublet, $J = 7.0$, two $-CH_3$ groups);

3.46 (1H, multiplet, $-CH<$ group);

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

5.46 (2H, Octet, $-CH_2-$ group in dioxaphosphorin ring);

7.1 (1H, doublet, one aromatic hydrogen meta to nitro group);

8.05 (1H, doublet, one aromatic hydrogen ortho to both nitro group and $-CH_2-$ group of dioxaphosphorin ring);

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

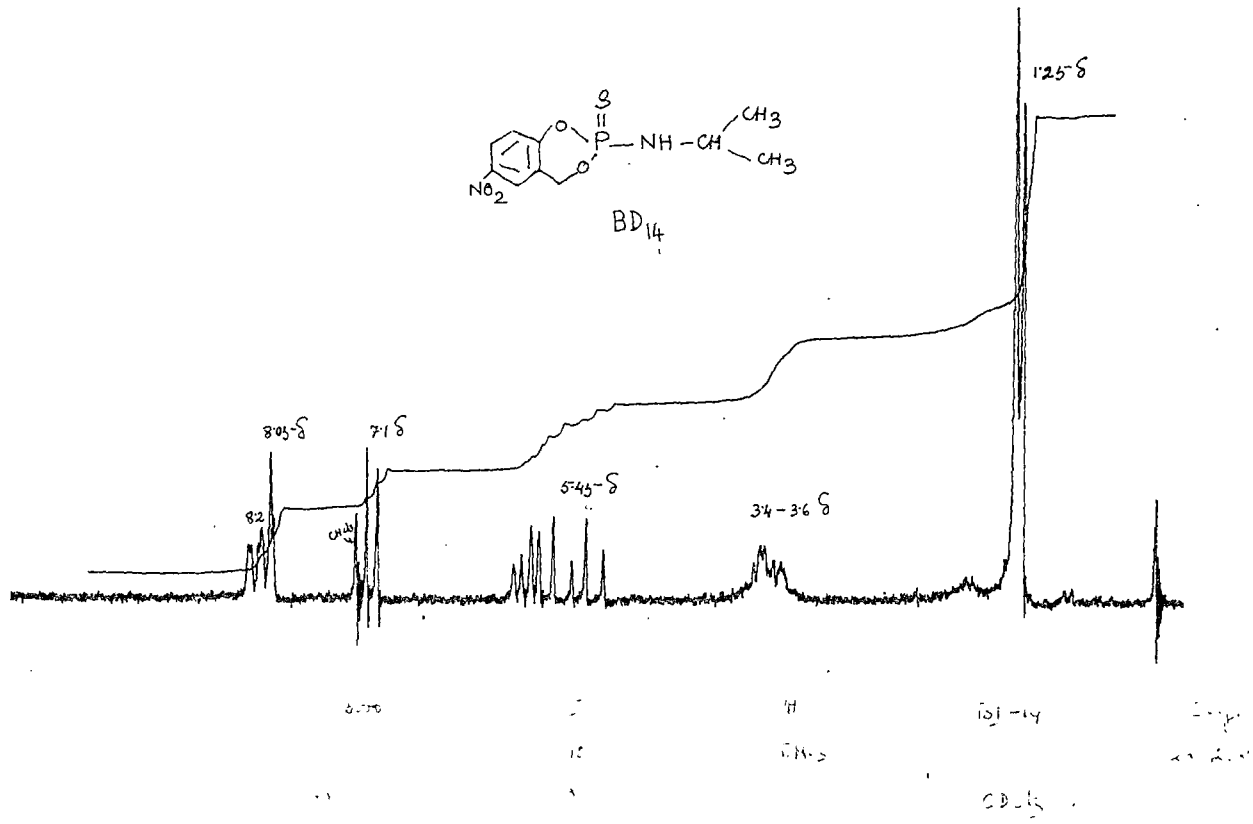


Fig. 23. ^1H NMR spectrum of 2- Isopropylamido- -6- nitro- 4H- 1, 3, 2- benzodioxaphosphorin -2- sulphide

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

IR (Fig.24):

1030 Cm^{-1} (s), P-O-C (alkyl);
1250 Cm^{-1} (s) and 875 Cm^{-1} (s), P-O-C (aryl);
1520 Cm^{-1} (vs), asym. str. of nitro group;
1340 Cm^{-1} (vs), sym. str. of nitro group;
810 Cm^{-1} (s), P = S (I);
655 Cm^{-1} (s), P = S (II);
735 Cm^{-1} (m), P - N str.

Mass (Fig. 25):

m/e	%RI
300 (M^+)	21.4
267	100
198	25.7
148	11.4
116	80.0
70	35.7

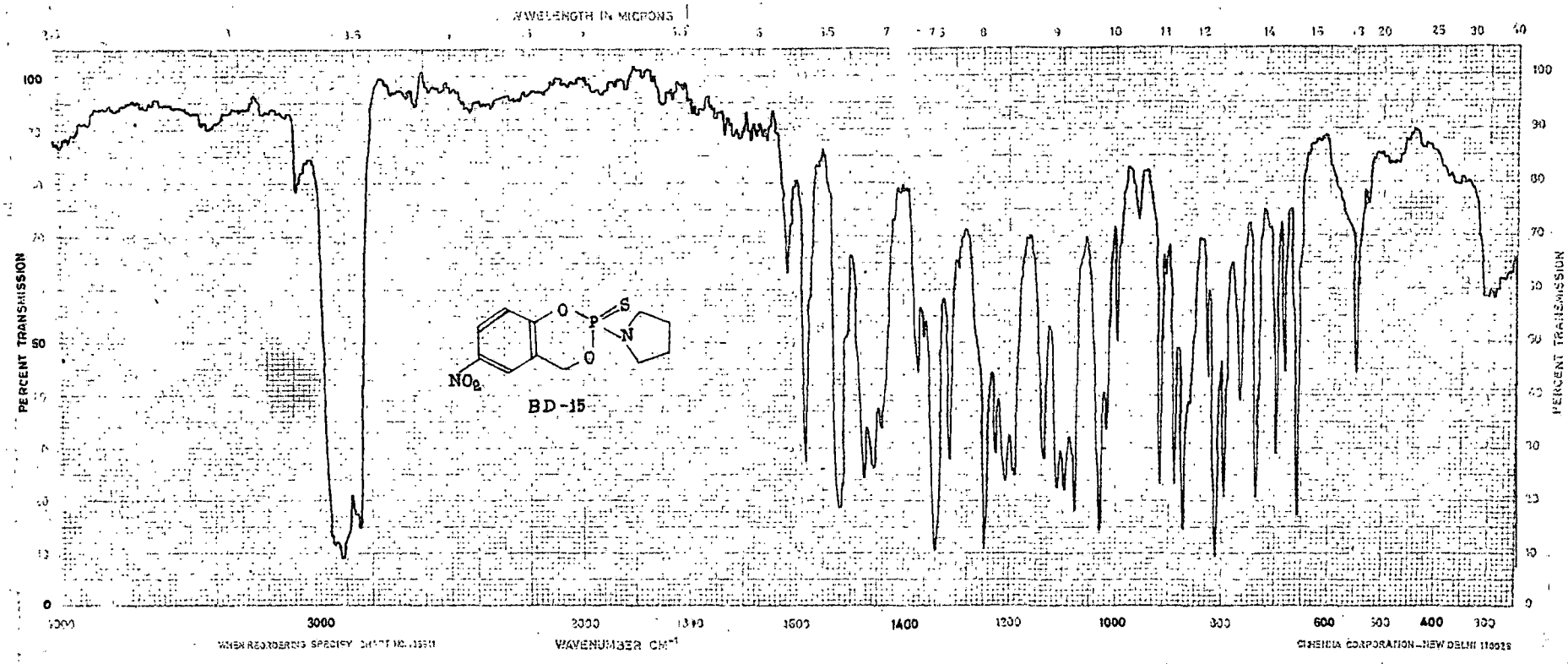
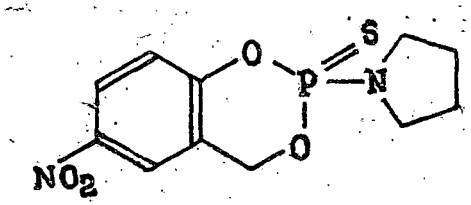


Fig. 24. IR spectrum of 2- Pyrrolidino - 6- nitro - 4H - 1,3,2- benzodioxaphosphorin - 2 - sulphide.

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

ANALYSE: 62907
==========

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



BD-15

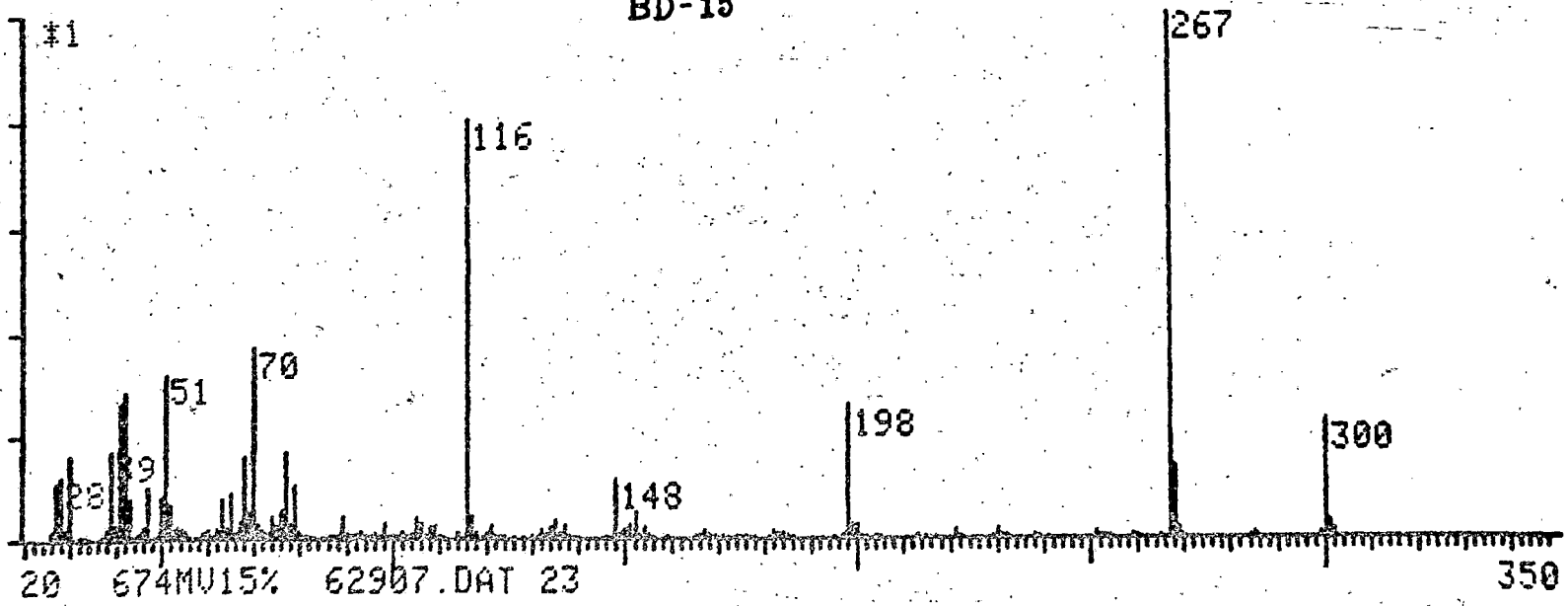


Fig. 25. Mass spectrum of 2-Pyrrolidino-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-sulphide

^1H NMR $\delta(CDCl_3)$ ppm (Fig. 26):

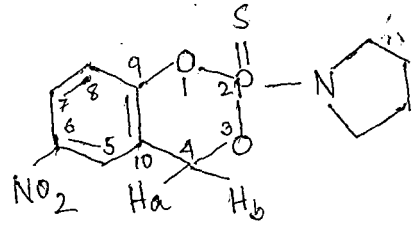
1.62 - 1.95 (4H, multiplet, two $-CH_2-$ groups at 2,2' positions of the pyrrolidine ring);

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

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

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

^{13}C NMR $\delta(CDCl_3)$ ppm (Fig. 27a - 27c):



Carbon atom	δ(ppm)	J(Hz)
$C_{2'}$	26.29	$^3J_{P-N-C_{1'}-C_{2'}}$ = 9.87
$C_{1'}$	47.68	$^2J_{P-N-C_{1'}}$ = 6.83
C_4	65.62	$^2J_{P-O-C_4}$ = 5.53
C_5	121.29	
C_{10}	121.51	$^3J_{P-O-C_9-C_{10}}$ = 12.38
C_7	124.81	$^4J_{P-O-C_9-C_8-C_7}$ = 0.93
C_6	143.03	
C_9	155.64	$^2J_{P-O-C_9}$ = 7.3

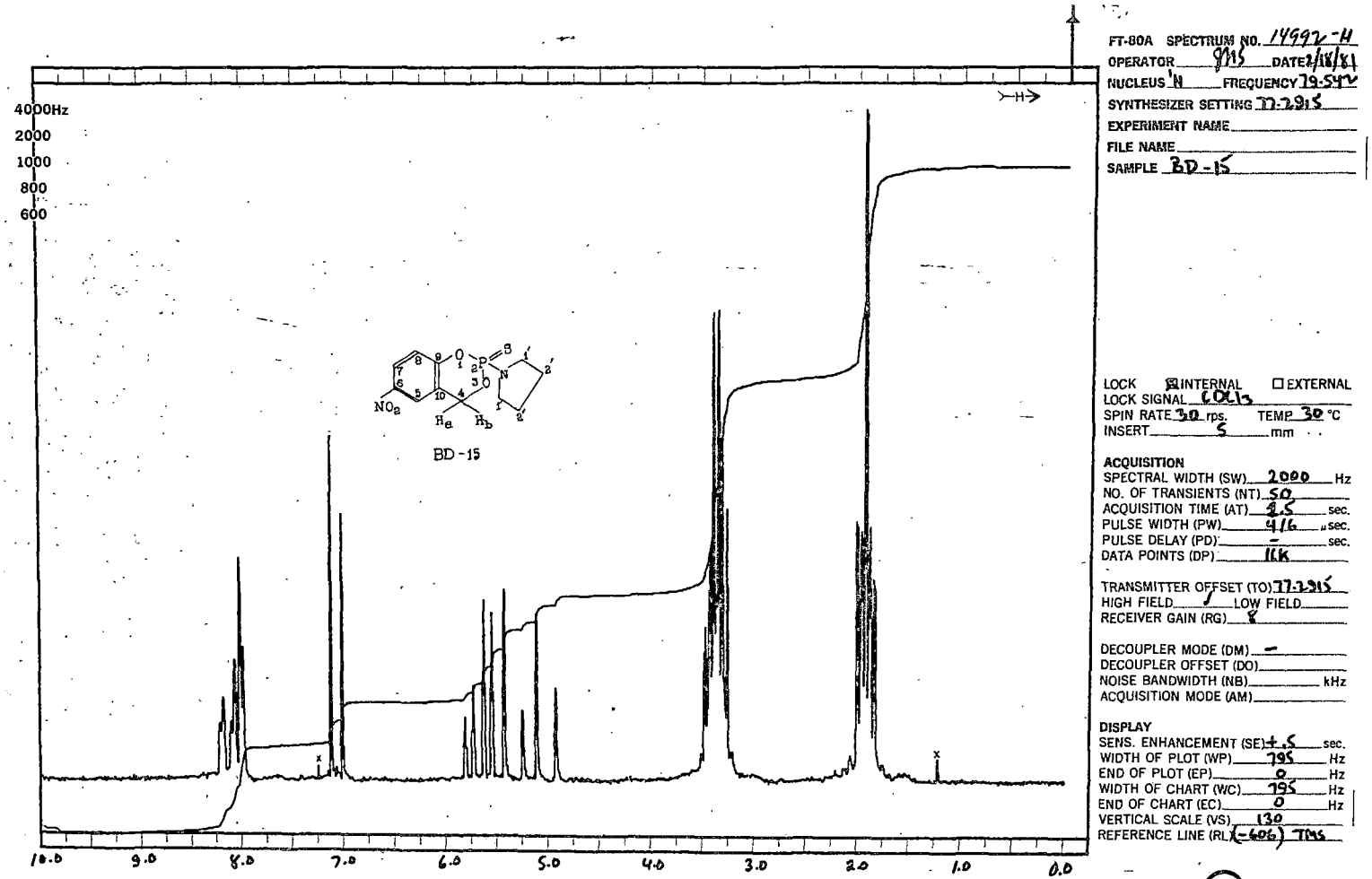
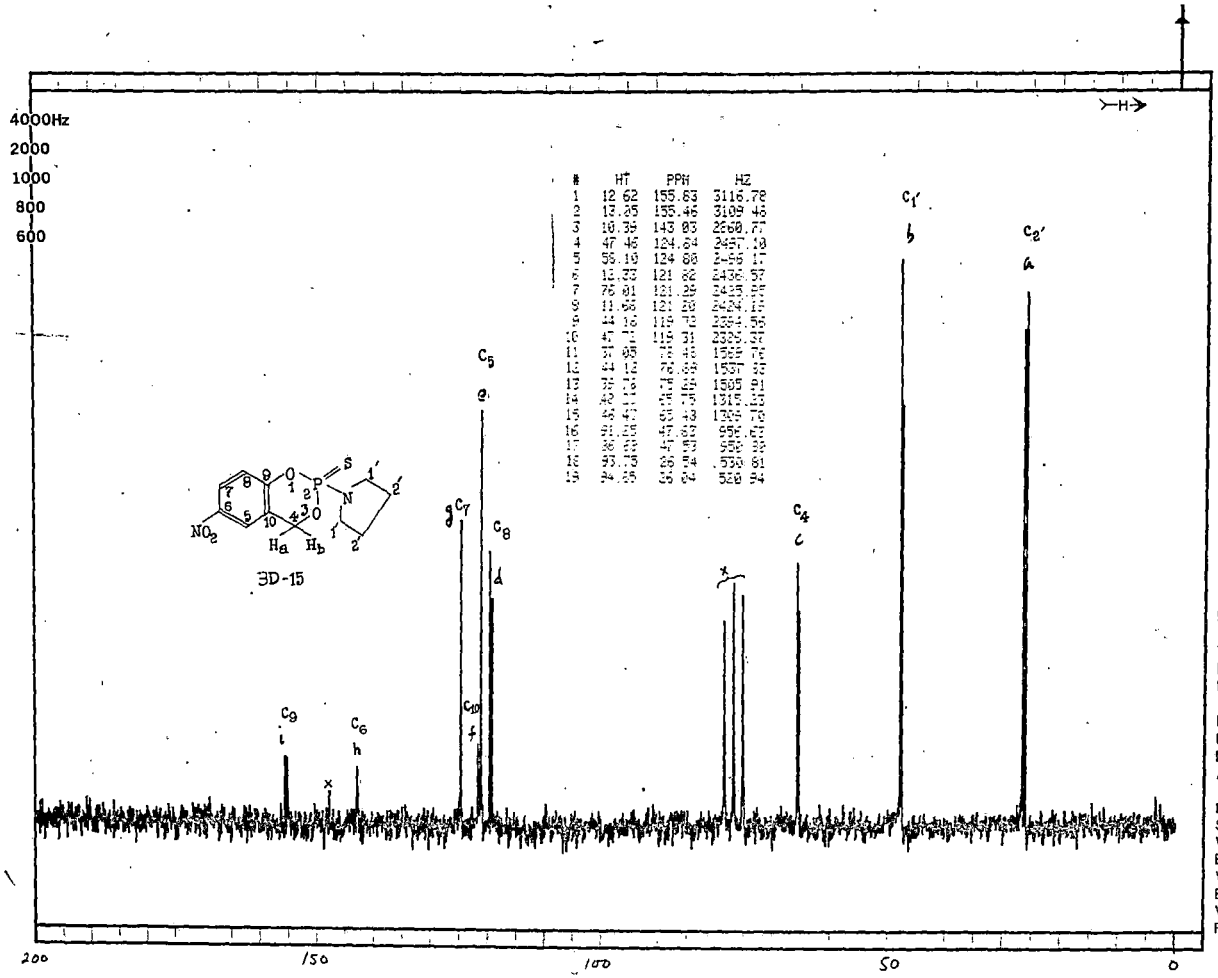


Fig. 26. ^1H NMR spectrum of 2-Pyrrolidino-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-sulphide



20·0 MHz proton noise-decoupled ^{13}C spectrum of BD-15 in $CDCl_3$

Fig. 27a

FT-80A SPECTRUM NO. 14992-6
OPERATOR GMS DATE 2/12/87
NUCLEUS ^{13}C FREQUENCY 20.0
SYNTHESIZER SETTING 17.7500
EXPERIMENT NAME
FILE NAME
SAMPLE BD-15

LOCK ☒ INTERNAL ☐ EXTERNAL
LOCK SIGNAL CDCl3
SPIN RATE 30 rps. TEMP 30 °C
INSERT 5 mm

ACQUISITION
SPECTRAL WIDTH (SW) 5000 Hz
NO. OF TRANSIENTS (NT) 11,000
ACQUISITION TIME (AT) 1.0 sec.
PULSE WIDTH (PW) 3/7 μsec.
PULSE DELAY (PD) sec.
DATA POINTS (DP) 16K

TRANSMITTER OFFSET (TO) 17.7500
HIGH FIELD ✓ LOW FIELD
RECEIVER GAIN (RG) 6

DECOUPLER MODE (DM) 1
DECOUPLER OFFSET (DO) 53
NOISE BANDWIDTH (NB) 2 kHz
ACQUISITION MODE (AM) 0

DISPLAY
SENS. ENHANCEMENT (SE) -2.0 sec.
WIDTH OF PLOT (WP) 4000 Hz
END OF PLOT (EP) 0 Hz
WIDTH OF CHART (WC) 4000 Hz
END OF CHART (EC) 0 Hz
VERTICAL SCALE (VS) 100
REFERENCE LINE (RL) 6682 TMS

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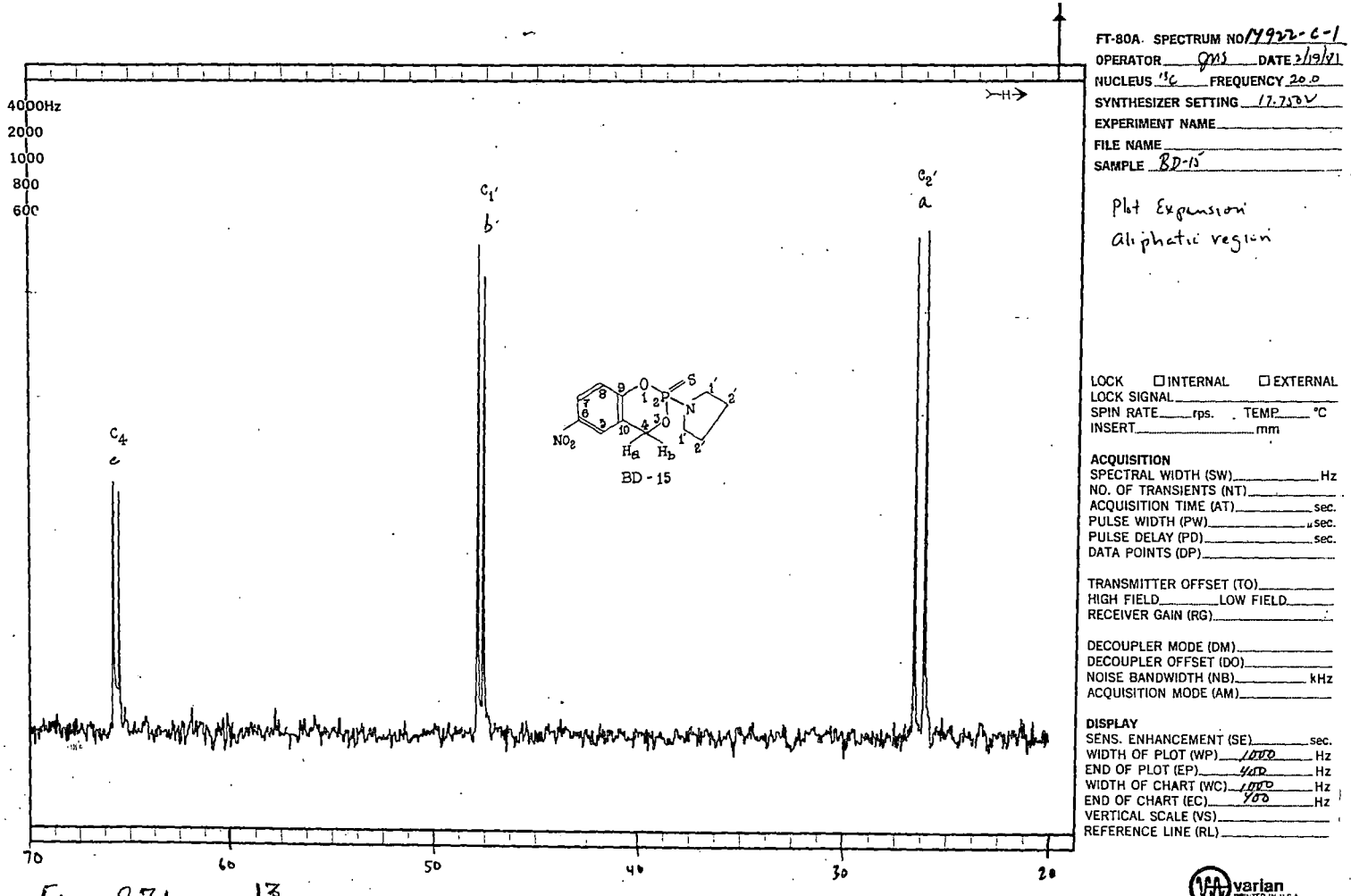

FT-80A. SPECTRUM NO 7922-C-1
OPERATOR QNS DATE 2/19/81
NUCLEUS ^{13}C FREQUENCY 20.0
SYNTHESIZER SETTING 17.750V
EXPERIMENT NAME
FILE NAME
SAMPLE BD-15

Plot Expansion
Aliphatic region

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

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

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

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

DISPLAY
SENS. ENHANCEMENT (SE) sec.
WIDTH OF PLOT (WP) 1000 Hz
END OF PLOT (EP) 400 Hz
WIDTH OF CHART (WC) 1000 Hz
END OF CHART (EC) 900 Hz
VERTICAL SCALE (VS)
REFERENCE LINE (RL)

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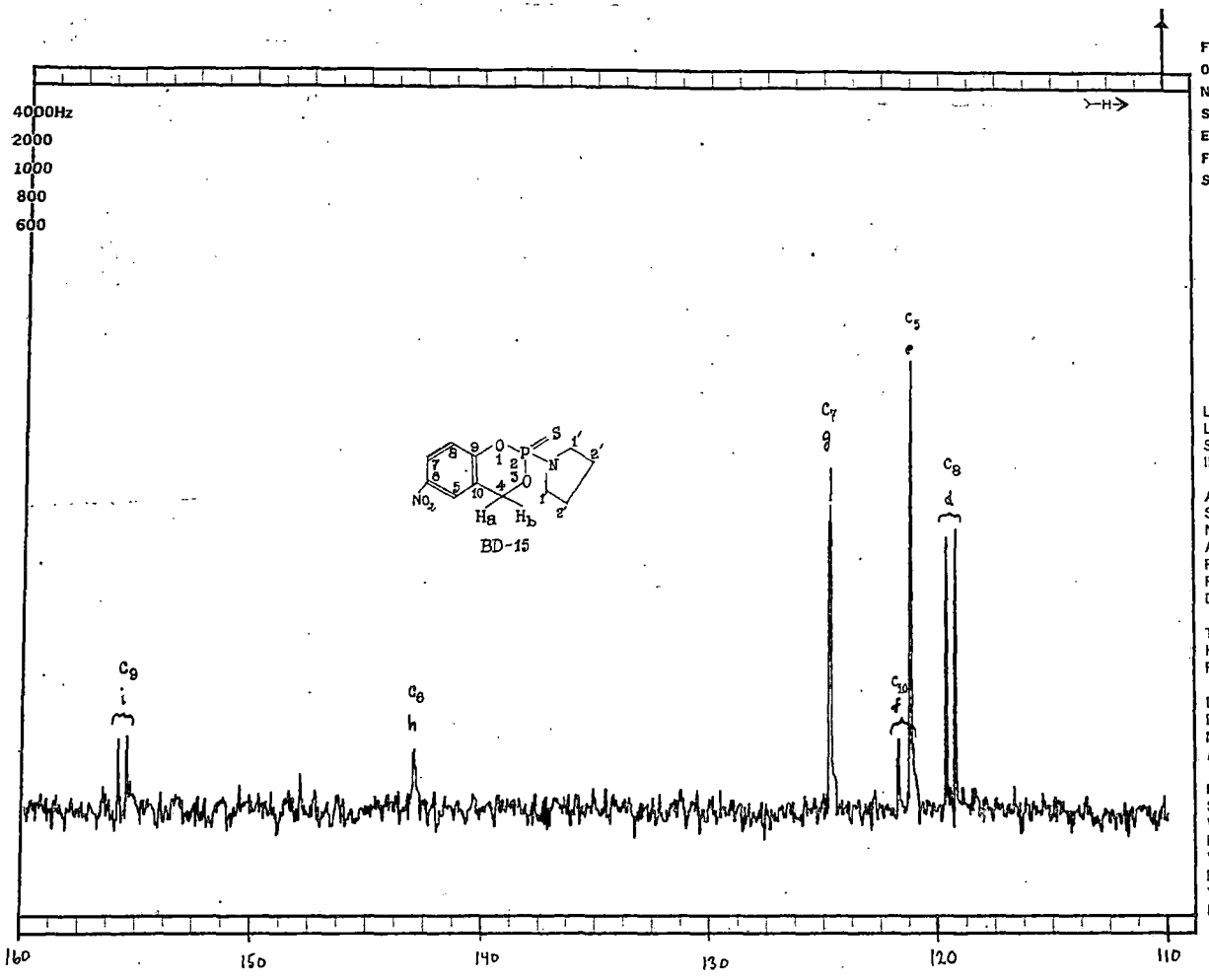
Fig. 27b. ^{13}C NMR spectrum of BD-15 (Plot expansion: Aliphatic region)



FT-80A SPECTRUM NO. 14522-C-2
OPERATOR GMS DATE 7/19/81
NUCLEUS ^{13}C FREQUENCY 20.0
SYNTHESIZER SETTING 17.7507
EXPERIMENT NAME
FILE NAME
SAMPLE BD-15

Plot Expansion
Aromatic region

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

ACQUISITION
SPECTRAL WIDTH (SW) 5000 Hz
NO. OF TRANSIENTS (NT) 11,000
ACQUISITION TIME (AT) 1.0 sec.
PULSE WIDTH (PW) 3/7 μsec.
PULSE DELAY (PD) – sec.
DATA POINTS (DP) 16K

TRANSMITTER OFFSET (TO) 17.750
HIGH FIELD ✓ LOW FIELD
RECEIVER GAIN (RG) 8

DECOUPLER MODE (DM) 1
DECOUPLER OFFSET (DO) 53
NOISE BANDWIDTH (NB) -2 kHz
ACQUISITION MODE (AM) 0

DISPLAY
SENS. ENHANCEMENT (SE) -2.0 sec.
WIDTH OF PLOT (WP) 1000 Hz
END OF PLOT (EP) 7500 Hz
WIDTH OF CHART (WC) 1000 Hz
END OF CHART (EC) 7500 Hz
VERTICAL SCALE (VS) 100
REFERENCE LINE (RL) (-682) TMS

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Fig. 27c. ^{13}C NMR spectrum of BD-15 (Plot expansion: Aromatic region)

(vii) 2-Piperidino-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-sulphide (BD-16):

IR (Fig. 28):

1025 Cm^{-1} (s), P-O-C (alkyl);
1245 Cm^{-1} (s) and 870 Cm^{-1} (s), P-O-C (aryl);
1515 Cm^{-1} (s), asym. str. of nitro group;
1340 Cm^{-1} (s), sym. str. of nitro group;
820 Cm^{-1} (s), P = S (I);
640 Cm^{-1} (m), P = S (II);
740 Cm^{-1} (s), P - N str.

Mass (Fig. 29):

m/e	%RI
314 (M^+)	17.1
281	100
198	7.1
130	82.9
84	12.9

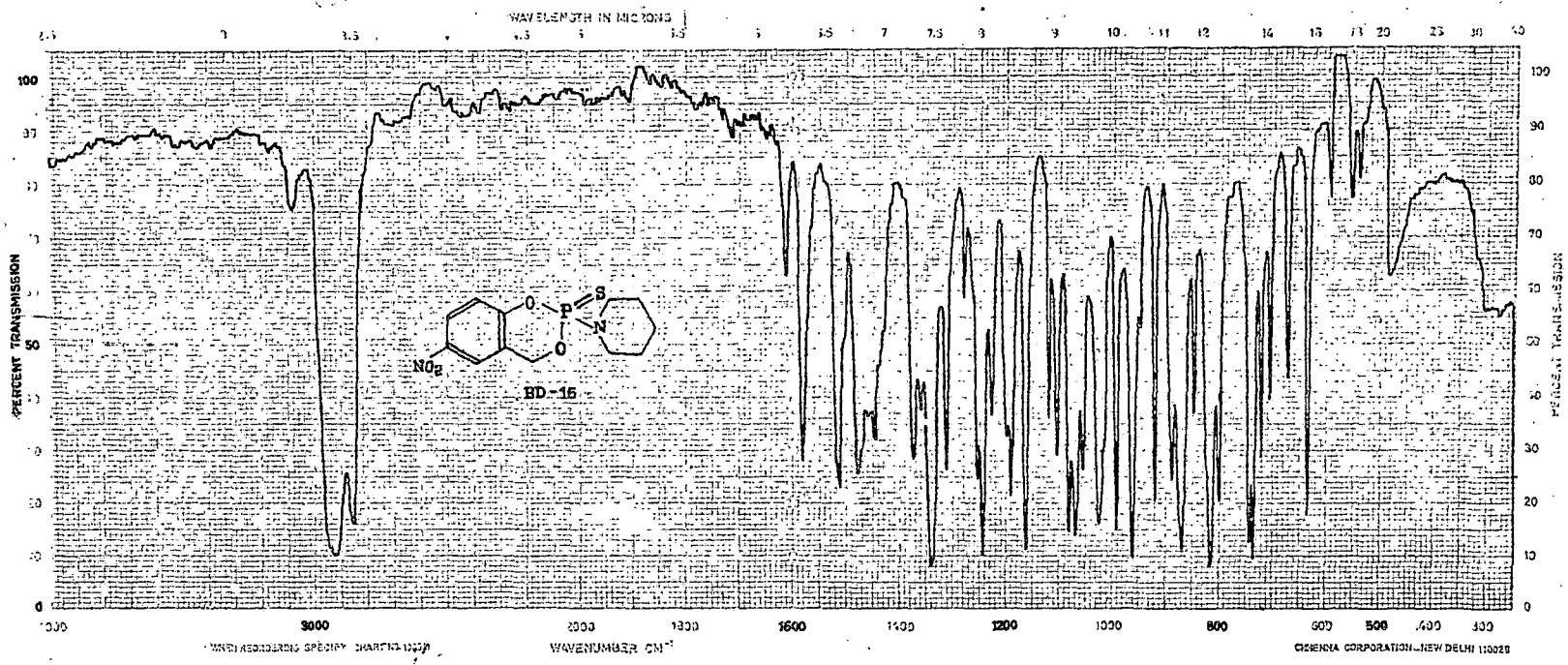
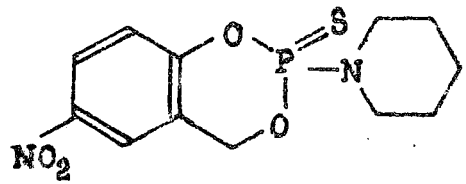


Fig. 28. IR spectrum of 2- Piperidino - 6- nitro - 4H - 1,3,2- benzodioxaphosphorin - 2- sulphide

SPEKTRUM 25 VERDAMPFUNGSTEMPERATUR 110 GRAD
MOLEKUELPEAK: 314
MASSEN CHARAKTERISTISCHER IONEN:
281=314-SH

ANALYSE: 62893

STH HA 016 00
ST STEENKEN
MESSG:
AUSW :25-MAR-81
AUSWER: SCH



BD-16

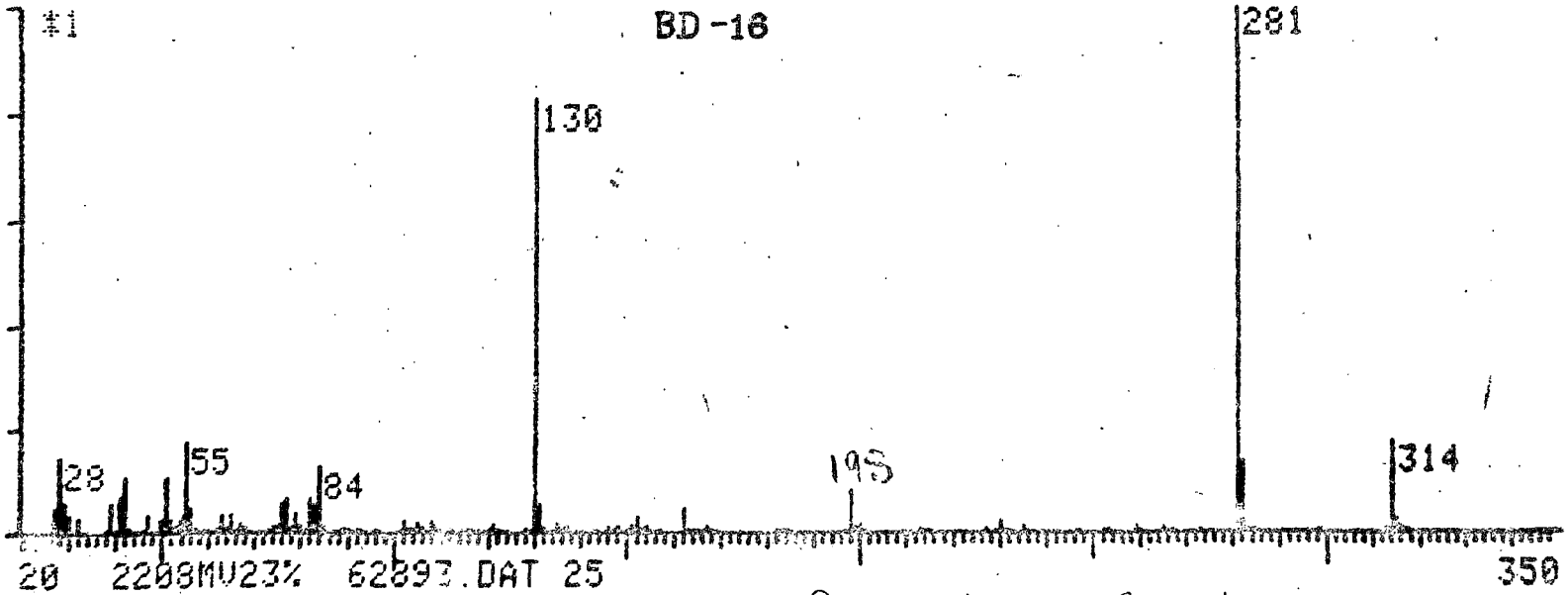


Fig. 29. Mass spectrum of 2-Piperidino-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-sulphide

^1H NMR δ ($CDCl_3$) ppm (Fig.30):

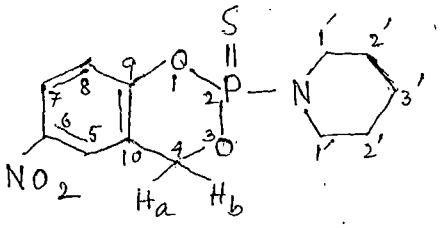
1.6 (6H, 3 $-CH_2-$ groups at 2',2' 3' positions of the piperidine ring);

3.15 - 3.5 (4H, multiplet, two $-CH_2-$ groups adjacent to nitrogen);

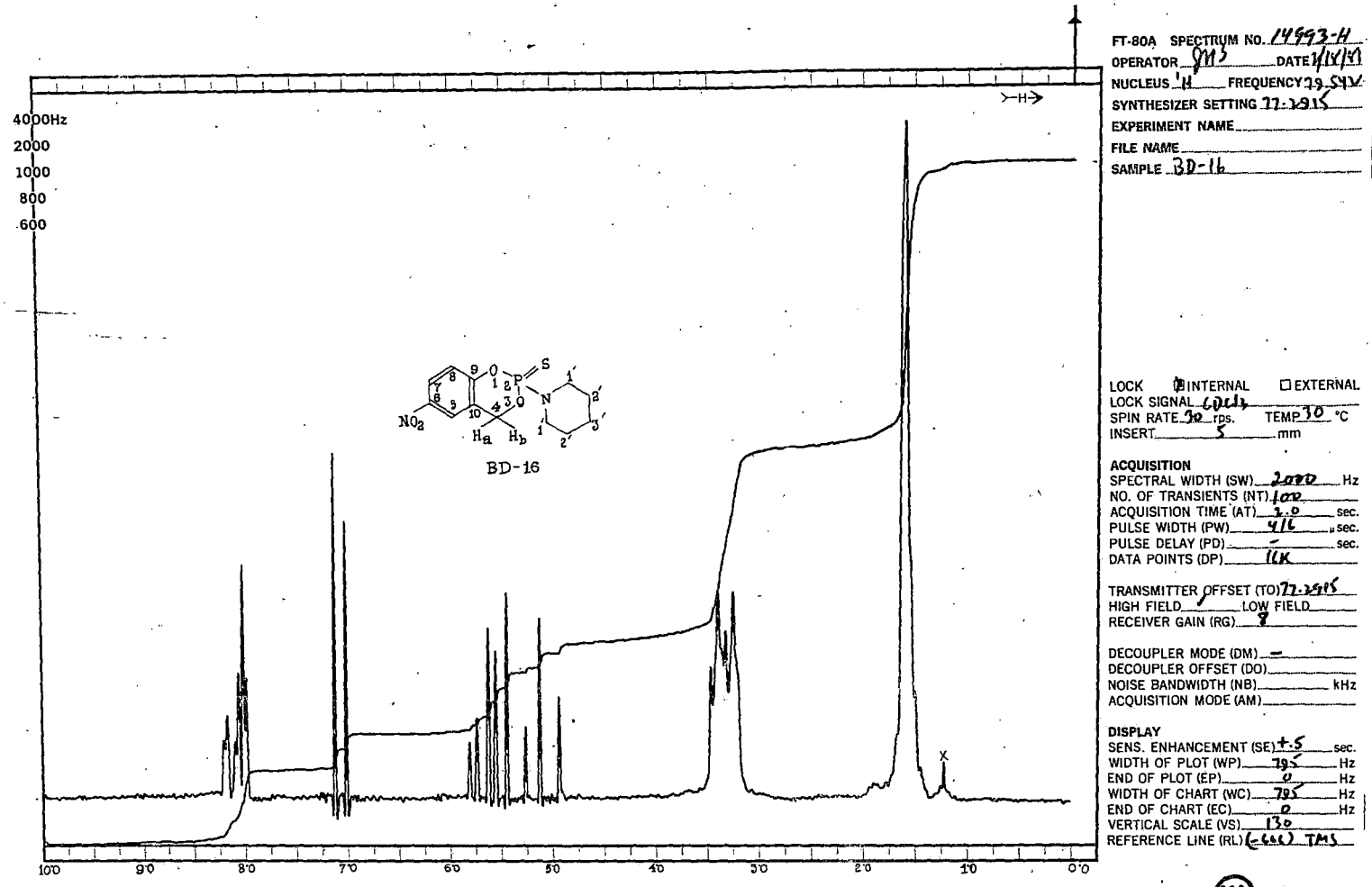
5.2 - 5.65 (2H, $-CH_2-$ group in dioxaphosphorin ring);

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

^{13}C NMR δ ($CDCl_3$) ppm (Fig. 31a - 31b):



Carbon atom	δ (ppm)	J(Hz)	
C_3,	24.1	$^4J_{P-N-C_1',-C_2',-C_3'}$	=1.2
C_2,	25.93	$^3J_{P-N-C_1',-C_2'}$	=3.7
C_1,	46.25	$^2J_{P-N-C_1'}$	=3.6
C_4	66.11	$^2J_{P-O-C_4}$	=5.75
C_8	119.63	$^3J_{P-O-C_9-C_8}$	=8.22
C_5	121.32		
C_{10}	121.36	$^3J_{P-O-C_9-C_{10}}$	=11.38
C_7	124.75		
C_6	145.06		
C_9	155.84	$^2J_{P-O-C_9}$	=7.0



FT-80A SPECTRUM NO. 14993-H
OPERATOR SMS DATE 7/14/91
NUCLEUS ^1H FREQUENCY 79.54V
SYNTHESIZER SETTING 77.2915
EXPERIMENT NAME
FILE NAME
SAMPLE BD-16

LOCK ■ INTERNAL □ EXTERNAL
LOCK SIGNAL CDCl3
SPIN RATE 30 rps. TEMP 30 °C
INSERT 5 mm

ACQUISITION
SPECTRAL WIDTH (SW) 2000 Hz
NO. OF TRANSIENTS (NT) 100
ACQUISITION TIME (AT) 1.0 sec.
PULSE WIDTH (PW) 4/6 μsec.
PULSE DELAY (PD) – sec.
DATA POINTS (DP) 16K

TRANSMITTER OFFSET (TO) 77.2915
HIGH FIELD / LOW FIELD
RECEIVER GAIN (RG) 8

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

DISPLAY
SENS. ENHANCEMENT (SE) +.5 sec.
WIDTH OF PLOT (WP) 795 Hz
END OF PLOT (EP) 0 Hz
WIDTH OF CHART (WC) 795 Hz
END OF CHART (EC) 0 Hz
VERTICAL SCALE (VS) 130
REFERENCE LINE (RL) (-446) TMS

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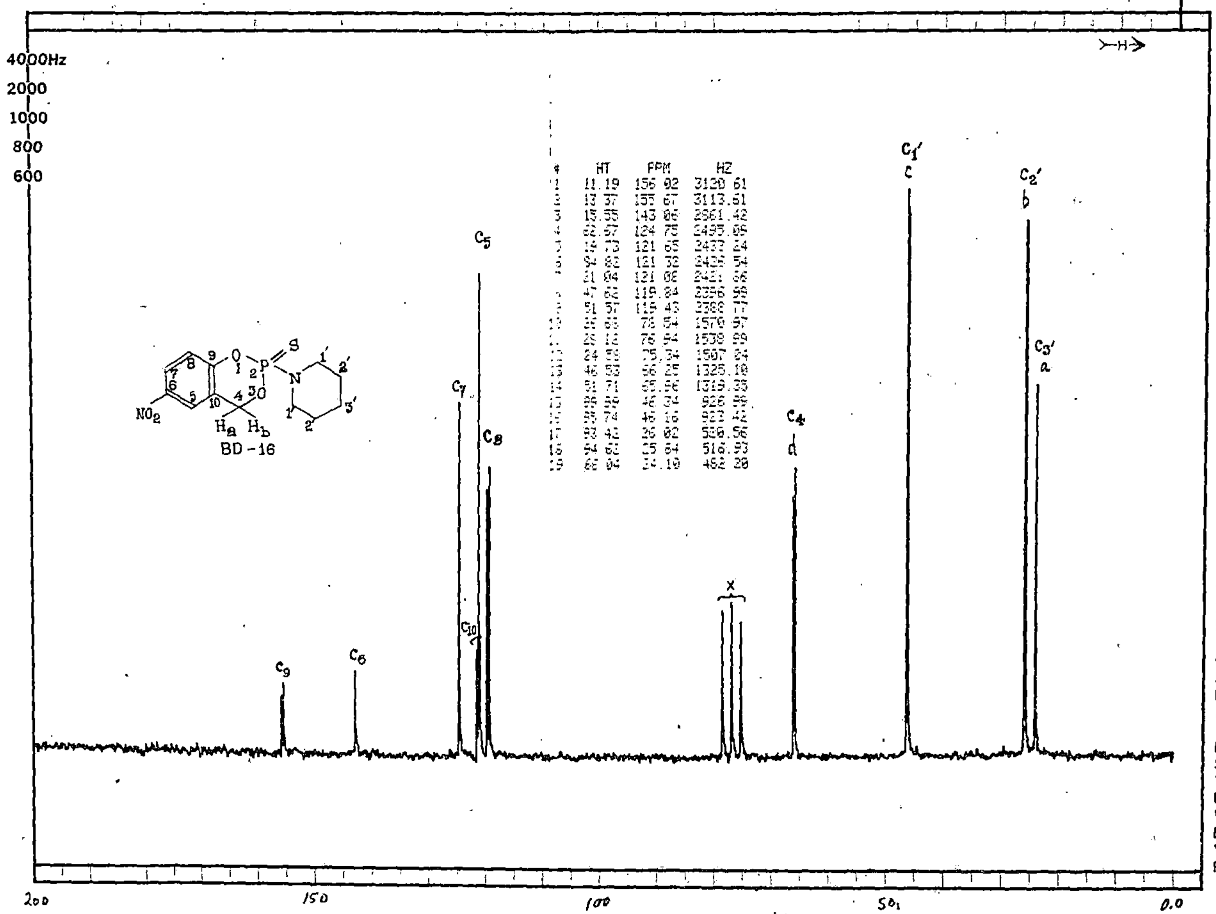
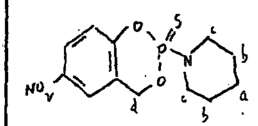
Fig. 30: ^1H NMR spectrum of 2-Piperidino-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-sulphide.



#	HT	PPM	HZ
1	11.19	156.02	3120.61
2	13.37	155.67	3113.51
3	15.55	143.06	2861.42
4	62.57	124.75	2495.05
5	19.73	121.65	2433.24
6	94.82	121.32	2426.54
7	21.04	121.06	2421.66
8	47.62	119.84	2396.99
9	51.57	115.43	2308.77
10	26.63	78.54	1570.97
11	28.12	76.94	1538.99
12	24.59	75.34	1507.04
13	46.53	66.25	1325.10
14	51.71	65.96	1319.33
15	35.59	46.34	926.99
16	35.74	46.16	923.42
17	93.42	26.02	520.56
18	94.62	25.84	516.93
19	62.04	24.10	482.20

FT-80A SPECTRUM NO. 14993-C
OPERATOR gns DATE 2/19/81
NUCLEUS 13C FREQUENCY 20.0
SYNTHESIZER SETTING 17.750V
EXPERIMENT NAME
FILE NAME
SAMPLE BD-16



LOCK ☒ INTERNAL ☐ EXTERNAL
LOCK SIGNAL CDCl3
SPIN RATE 30 rps. TEMP 30 °C
INSERT 5 mm

ACQUISITION
SPECTRAL WIDTH (SW) 5000 Hz
NO. OF TRANSIENTS (NT) 55409
ACQUISITION TIME (AT) 1.0 sec.
PULSE WIDTH (PW) 3/7 μsec.
PULSE DELAY (PD) – sec.
DATA POINTS (DP) 1LK

TRANSMITTER OFFSET (TO) 17.750V
HIGH FIELD ✓ LOW FIELD
RECEIVER GAIN (RG) 8

DECOUPLER MODE (DM) 1
DECOUPLER OFFSET (DO) 53
NOISE BANDWIDTH (NB) -2 kHz
ACQUISITION MODE (AM) 0

DISPLAY
SENS. ENHANCEMENT (SE) -1.0 sec.
WIDTH OF PLOT (WP) 4000 Hz
END OF PLOT (EP) 0 Hz
WIDTH OF CHART (WC) 4000 Hz
END OF CHART (EC) 0 Hz
VERTICAL SCALE (VS) 100
REFERENCE LINE (RL) (-652) TMS

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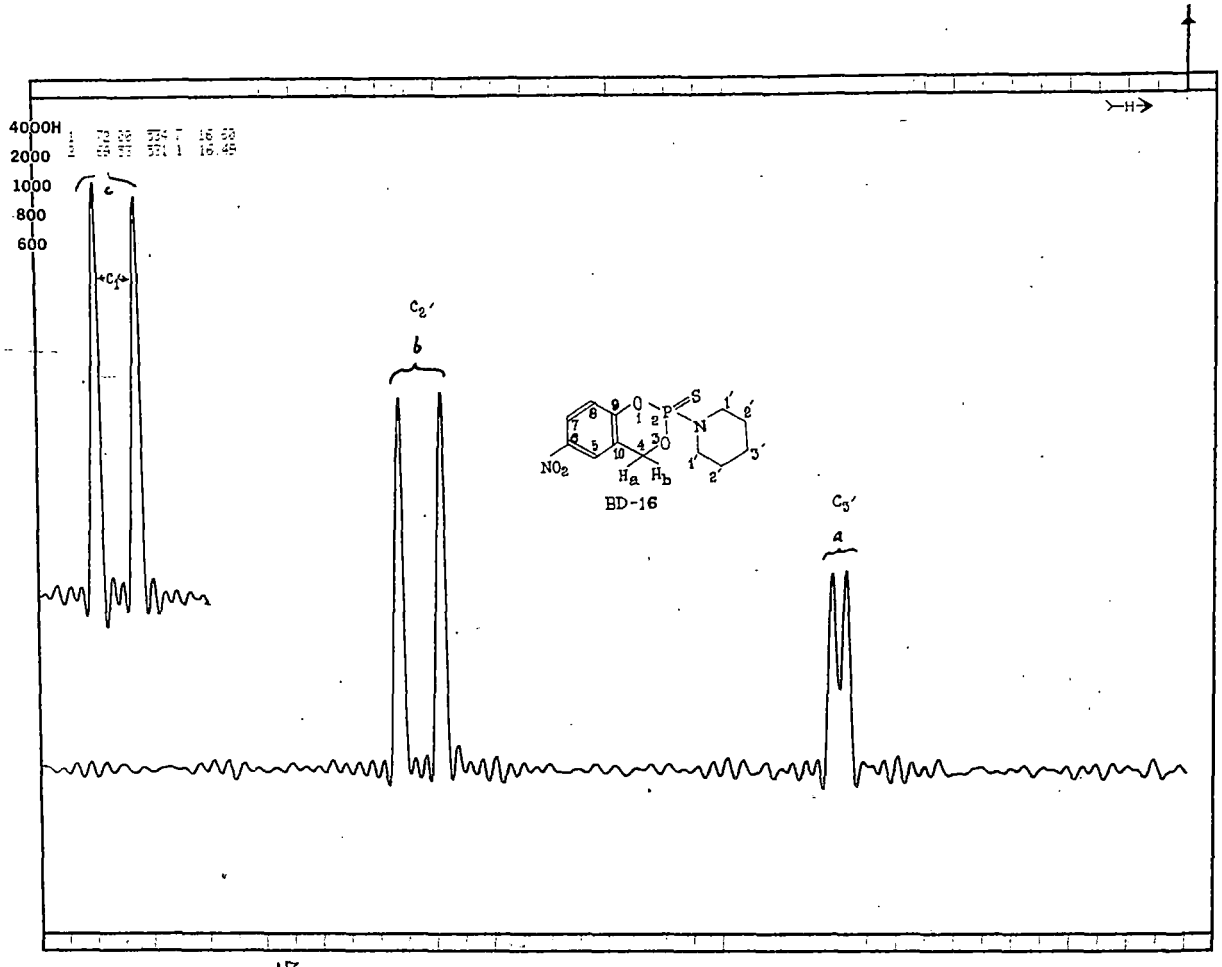
20.0 MHz proton noise-decoupled ^{13}C spectrum of BD-16 in $CDCl_3$

Fig- 31a



FT-80A SPECTRUM NO. 14993-c-1
OPERATOR Jms DATE 7/19/81
NUCLEUS FREQUENCY
SYNTHESIZER SETTING
EXPERIMENT NAME
FILE NAME
SAMPLE BD-16
Plot Expansion.

1	65.35	128.3	3.98
2	66.29	124.6	3.87
3	34.96	98.5	2.81
4	35.42	69.3	2.77

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

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

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

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

DISPLAY
SENS. ENHANCEMENT (SE) +0.3 sec.
WIDTH OF PLOT (WP) 100 Hz
END OF PLOT (EP) Hz
WIDTH OF CHART (WC) 100 Hz
END OF CHART (EC) Hz
VERTICAL SCALE (VS) 72
REFERENCE LINE (RL)

varian PRINTED IN U.S.A.

Fig. 31.b.: ^{13}C NMR spectrum of BD-16 (Plot expansion)

(viii) 2-Nonylamido-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-sulphide (BD-17):

IR (Fig. 32):

1030 Cm^{-1} (s), P-O-C (alkyl);
1250 Cm^{-1} (s) and 880 - 895 Cm^{-1} (s), P-O-C (aryl);
1520 Cm^{-1} (s), asym. str. of nitro group;
1340 Cm^{-1} (s), sym. str. of nitro group;
810 Cm^{-1} (s), P = S (I);
660 Cm^{-1} (s), P = S (II);
3310 Cm^{-1} (s), N - H str.;
1620 Cm^{-1} (w) and 1585 Cm^{-1} (m), two components of the substituted benzene ring "quadrant stretching" C = C vibrations;
740 Cm^{-1} (m), P - N str. (also aromatic C-H-O bending).

Mass (Fig. 33):

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

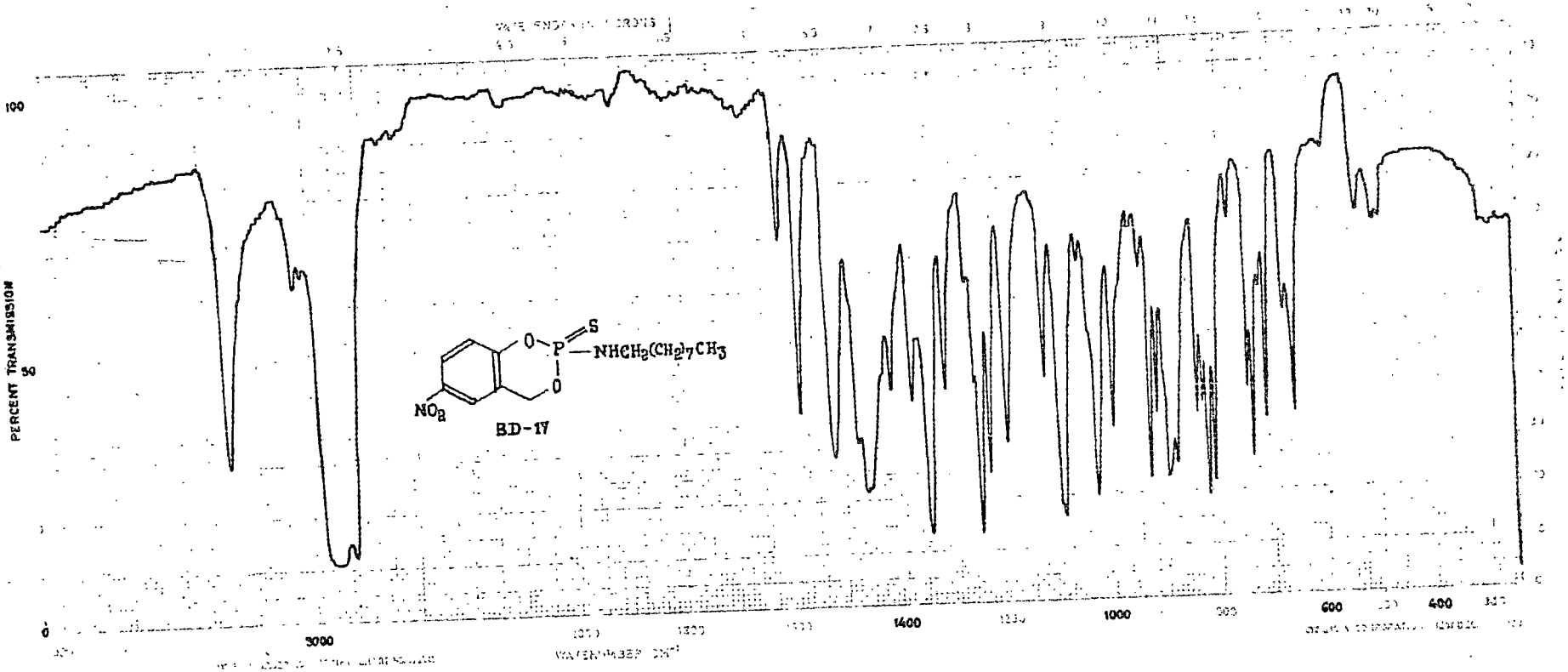


Fig. 32. IR spectrum of 2- Nonylamido – 6 - nitro – 4H - 1,3,2 - benzodioxaphosphorin – 2 - sulphide

SPEKTRUM 15 VERDAMPFUNGSTEMPERATUR 140 GRAD
MOLEKULARPEAK: 372
MASSEN CHARAKTERISTISCHER IONEN:
[illegible]-SH

ANALYSE: 62895

STN HA 017 : 0
ST.STEENKEN
MESSG:
AUSW :25-MAR-81
AUSWER: SCH

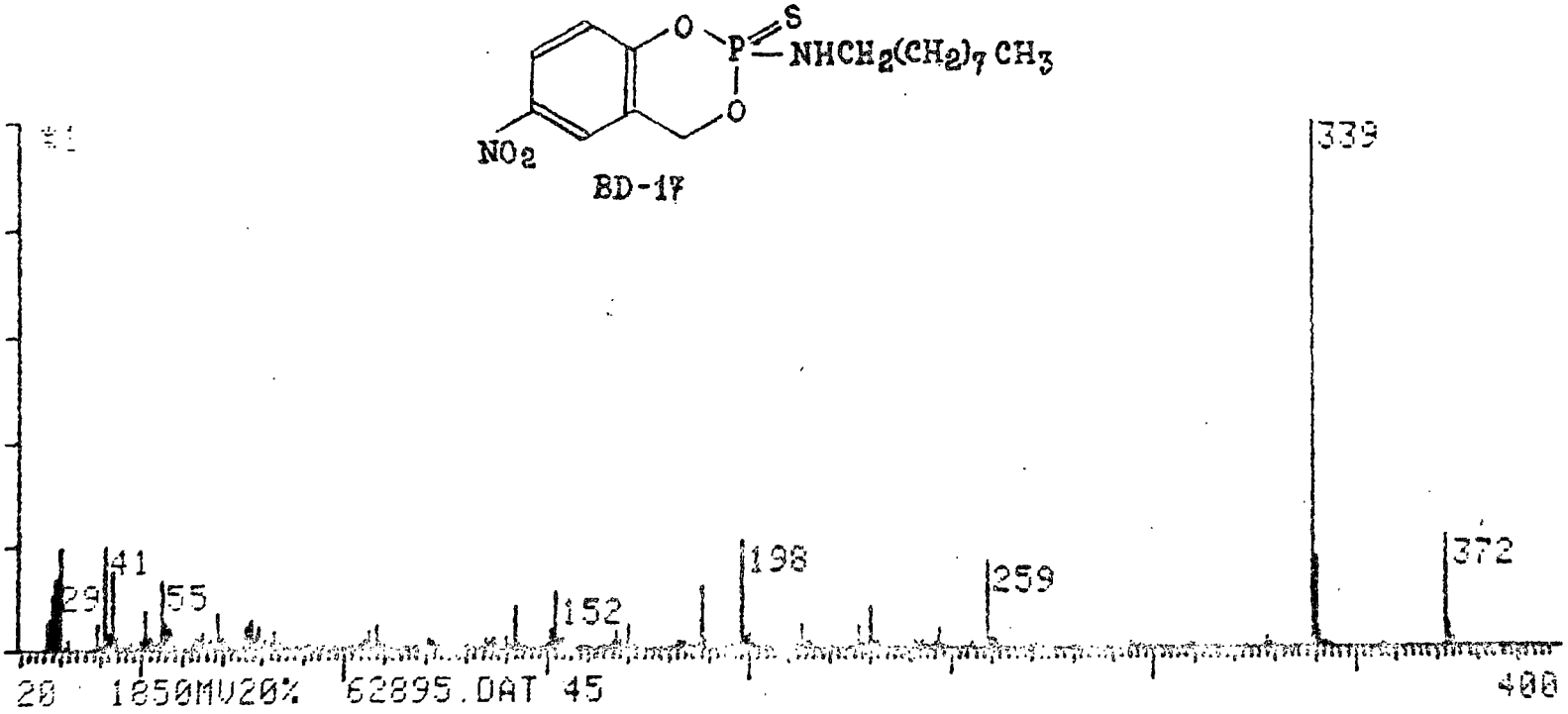


Fig. 33. Mass spectrum of 2-Nonylamido-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-sulphide

4. INTERPRETATION OF IR SPECTRA:

The IR spectra of the nitro salicenin cyclic phosphoramidothionates have been analysed according to Thomas [17], Bellamy [18], Coltrup et al [19] and Das [20]. The common IR bands for all compounds are summarised below:

1010 - 1030 cm^{-1} (s), P-O-C (alkyl);

1235 - 1250 cm^{-1} (vs), P-O-C (aryl);

880 - 920 cm^{-1} (s), P-O-C (aryl);

1515 - 1530 cm^{-1} (s), asym. str. of nitro group;

1340 - 1345 cm^{-1} (s), sym. str. of nitro group;

800 - 830 cm^{-1}, P = S (I);

640 - 660 cm^{-1}, P = S (II);

The thiono group is characterised by two IR absorption bands with frequencies in the normal ranges given by Thomas [17], 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 salicenin cyclic alkoxy/phenoxy compounds, Das et al. [20] have observed the two bands in the region : 650 - 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 affected by substitution (alkyl amido, 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 reflect changes in the inductive properties of the substituents, and this is not unexpected if they do indeed arise from mixed modes. It has also been observed that the P = S (I) band is of medium intensity while the intensity of the P = S (II) band is variable. This has also been reported by Thomas (17).

The frequencies of the P - N band for alkylamidophosphorothionates are : 740 cm^{-1} (BD-10), 720 cm^{-1} (BD-11), 785 cm^{-1} (BD-12), 780 cm^{-1} (BD-13), 785 cm^{-1} (BD-14), 740 cm^{-1} (BD-16) and 740 cm^{-1} (BD-17) ; we could not identify the P - N bond frequency in the iso-propylamido compound (BD-15). Workers have great difficulty in assigning the vibration frequency of the P - N bond. Thomas (17) has reviewed the assignments and correlations which have been proposed by other workers, and summarizes these as showing that P - N-(X) group is characterized by two absorption bands with frequencies in the ranges 645 - 887 cm^{-1} and 802 - 1150 cm^{-1}. He then proceeds to discuss indirect correlations for certain groups containing P - N bonds and finally concludes that, as all these indirect correlations have medium to strong absorption bands in the frequency range 789 - 1102 cm^{-1}, and as no consistent absorption band has been detected at lower frequencies, then this is the most reliable band for the identification of a P - N bond in an unknown molecule. As the P - N link must be expected to be mass sensitive, and therefore, the P - N absorption should show considerable frequency shifts with minor alterations in structure. Thomas (17) has noted a number of examples in which the P - N-link shows no absorption in the range quoted and also our experience confirms this. The P - N bond absorption frequency in iso-propyla-

ride compound (ED-14) could not be identified.

Although P - N absorption shows considerable frequency shifts, the N - H stretching vibrations occur in the normal frequency region:

3300 cm^{-1} in ED - 10
3290 cm^{-1} in ED - 14 ; and
3310 cm^{-1} in ED - 17.

The P-O-C (alkyl) group is characterized by a strong absorption band whose frequency lies between 1010 - 1030 cm^{-1}, while the band due to P-O-C (aryl) group is found in the region : 1235 - 1250 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 separate P = O str. which is not so coupled. Thomas[17] strongly favours the latter explanation which is supported by the persistence of this band in both P-O-P and P-O-C 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 substituent groups, p. - 34, Ref. 17) of the substituents. This band lies between 880 - 920 cm^{-1} for all compounds; the frequency range quoted is that of the strongest band in this region. Although 1020 cm^{-1} band has been identified for the vibration of P-O-C (alkyl) group in the morpholino compound (ED-11), the 980 cm^{-1} band has strongest intensity in the 950 - 1050 cm^{-1} region. It may be possible that this 980 cm^{-1} band is due to P-O-C (alkyl) group vibration in the morpholino compound.

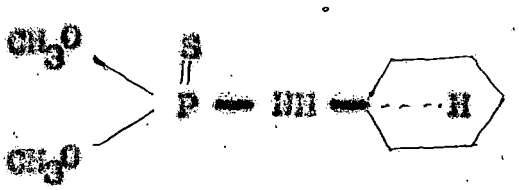
Two bands present in the ranges 1515 - 1520 cm^{-1} and 1340 - 1345 cm^{-1} are due to asym. and sym. str. of nitro group respectively (Bellamy, p. 335). The bands present at 1630 cm^{-1} and 1585 cm^{-1} are due to "quadrant stretching" C = C vibration of the aromatic ring (Colthup et al p. 224).

6. DISCUSSION ON MASS SPECTRA:

The mass spectra of these compounds have been analysed according to Cooks and Gerrard[22], Jörg et al[23], Ménico et al[24,25] and Gillis and Occolowitz[26].

All compounds show parent molecular ions. Fragmentation by loss of .SH radical is important; all compounds show an ion due to (parent molecule — SH)$^+$ and it is the base peak in spectra of cyclohexylamido (Fig. 6), morpholino (Fig. 10), diethylamido (Fig.14), pyrrolidino (Fig. 26), piperidino (Fig. 30), and morylamido (Fig.34) compounds; but m/e 198 is the base peak for both dimethylamido (Fig. 18) and isopropylamido (Fig.22) compounds.

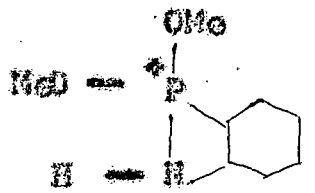
Cooks and Gerrard[22] reported that compounds of the type (A)



(A)

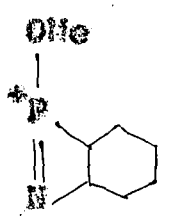
formed the base peak by loss of .SH radical directly from the molecular ion. By deuteration of the methyl group and the amino group

it was shown that the hydrogen of NH is abstracted from the cyclohexyl ring and not from the N - H entity. There was no preliminary hydrogen shift to sulphur. They postulated structure (B) for the product ion



(B)

By specific loss of the amino hydrogen, (B) further lost CH_3OH giving an ion for which they postulated structure (C)



(C)

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

Following Cooks and Gerrard[22], we postulate the mass fragmentation processes for the different nitro saligenin cyclic amidophosphorothionates.

Cyclohexylamido compound shows m/e 295 ion as the base peak by the direct elimination of NH from the molecular ion peak

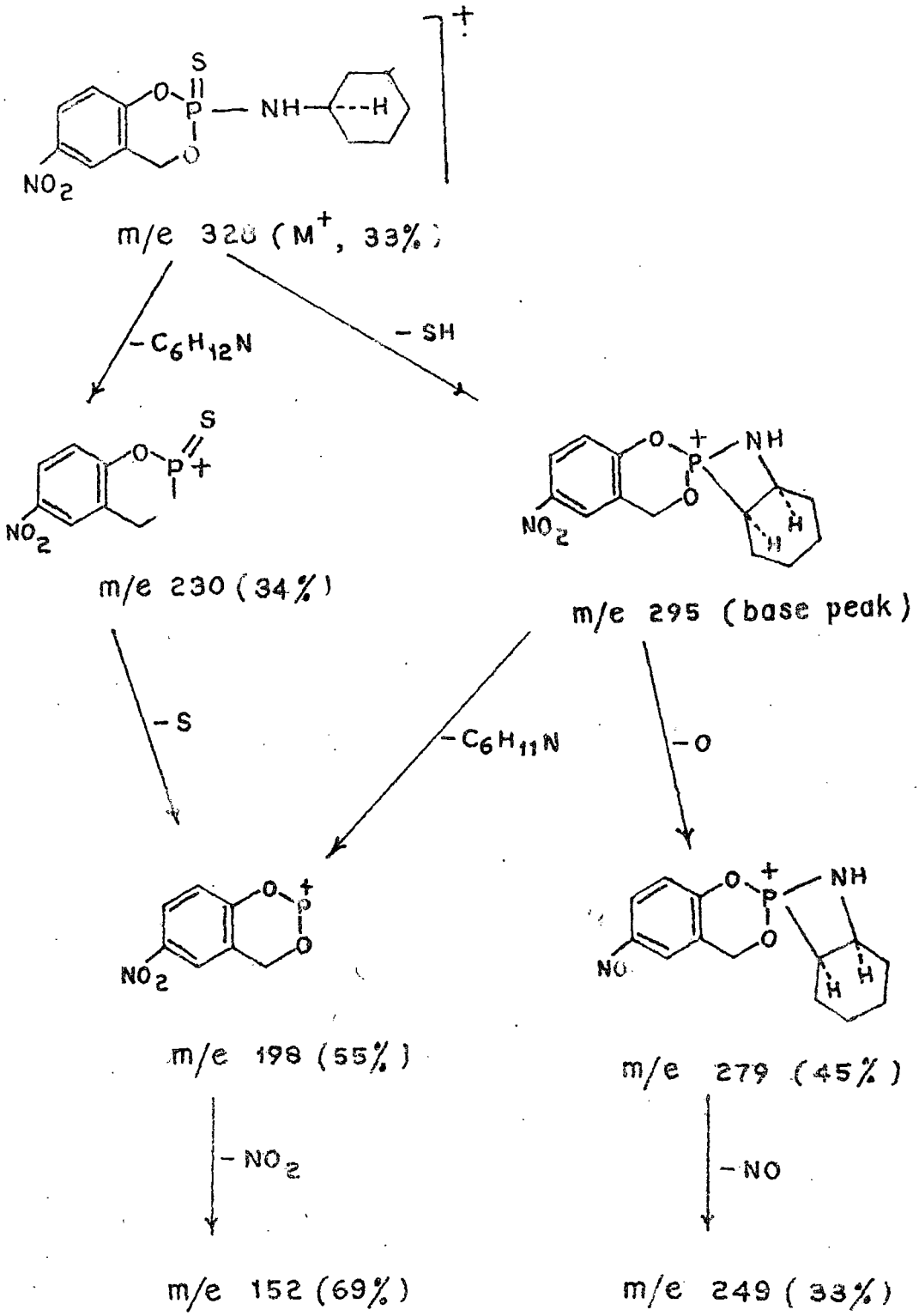
(m/e 333, % RI 33, Scheme-I). The ion (m/e 230, RI 34) is formed by the direct loss of $C_6H_{12}N$ from the molecular ion. The ion (m/e 198, RI 55) is formed by loss of S from the ion m/e 230 and also by the elimination of $C_6H_{11}N$ from the base peak ion. The ion (m/e 152, RI 69) is formed by the elimination of NO_2 from the ion m/e 198. The peak for m/e 270, RI 45 is observed due to the elimination of O from the base peak, and then the ion (m/e 249, RI 33) is formed by loss of NO from the ion, m/e 279.

In the case of morpholino compound the base peak shows for m/e 233 by the direct elimination of SH from the molecular ion peak (m/e 319, RI 43, Scheme-2). The ion (m/e 230, RI 43.6) and the ion (m/e 234, RI 60) are formed from the molecular ion peak by the direct loss of C_4H_8NO and S respectively. The ions (m/e 198, RI 70% and m/e 152, RI 45.4) have also been observed.

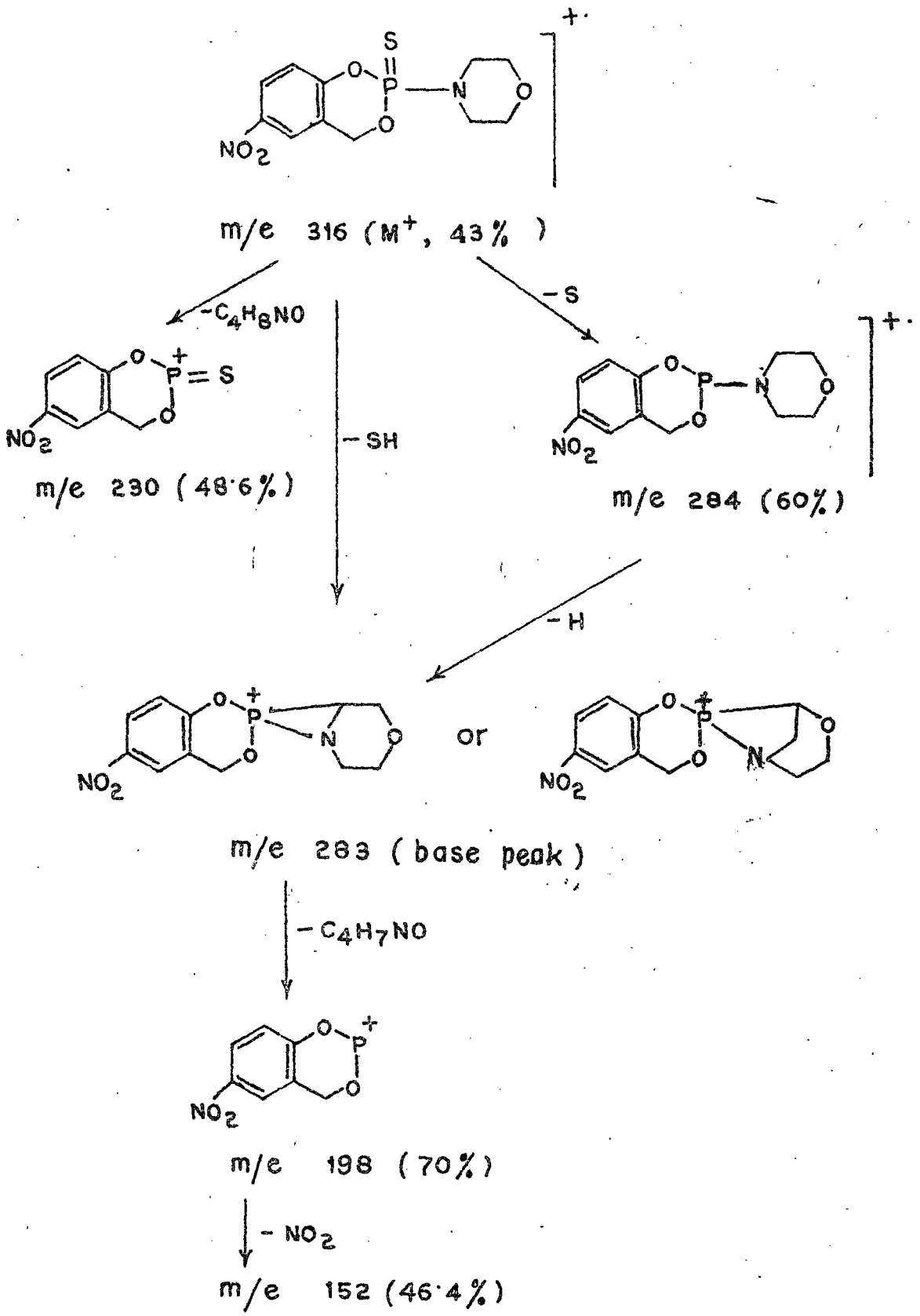
Diethylamido compound shows m/e 250 ion as the base peak by the direct elimination of SH from the molecular ion peak (m/e 302, RI 25, Scheme-3). The ions (m/e 198, RI 40 and m/e 152, RI 9.2) have also been observed like the other compounds.

In case of the dimethylamido compound the base peak ion is m/e 198 as mentioned earlier. The ion (m/e 241, RI 91.6) is observed by the direct loss of SH from the molecular ion. The ion (m/e 152, RI 9.2) has also been observed (Scheme-4).

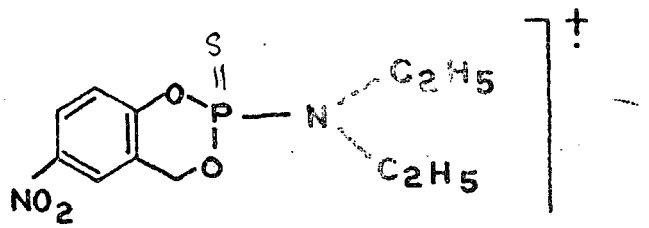
Isopropylamido compound also shows m/e 198 ion as the base peak. The ions (m/e 273, RI 25, m/e 255, RI 92 and m/e 230,



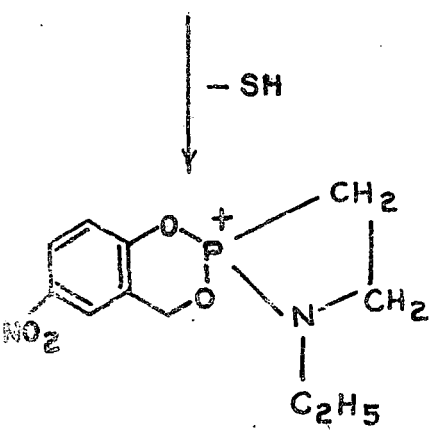
Scheme. Mass fragmentation of BD_{10}



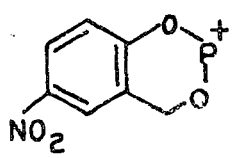
Scheme – Mass Fragmentation of BD_{11}



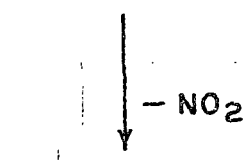
m/e 302 (M^+, 25%)



m/e 269 (base peak)

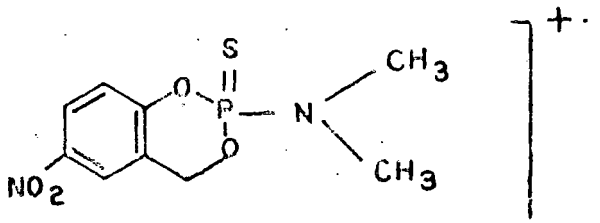


m/e 198 (40%)



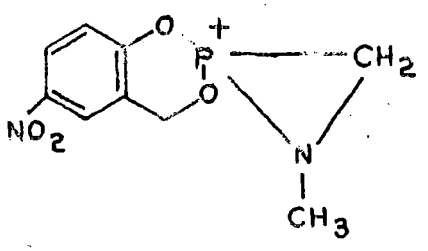
m/e 152 (9·2%)

Scheme. Mass Fragmentation of BD_{12}



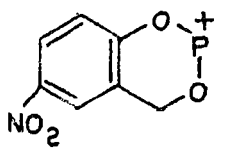
m/e 274 (M^+, 94·4%)

$\downarrow$ $-SH$



m/e 241 (91·6%)

$\downarrow$ $-C_2H_5N$



m/e 198 (base peak)

$\downarrow$ $-NO_2$

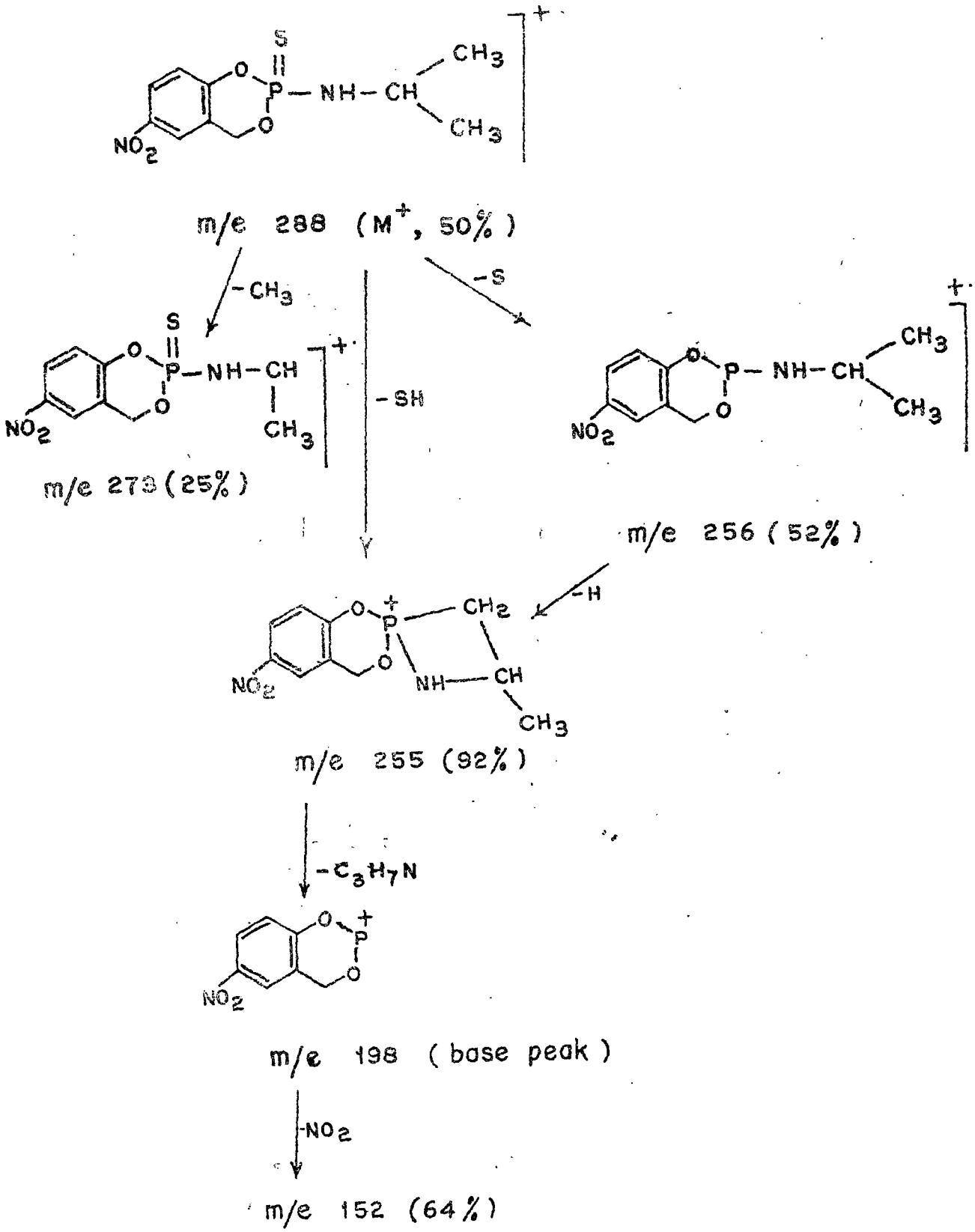
m/e 152 (9·2%)

RM 62) are observed by the direct loss of CH_3, OH and O respectively from the molecular ion peak. The ion m/e 255 may also be formed by the loss of H from the ion m/e 256. The ion (m/e 152, RM 64) is also observed as before (Scheme-5).

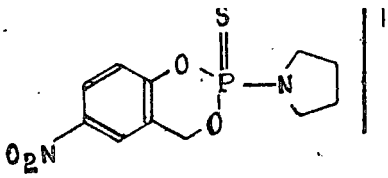
Pyrrolidine compound shows m/e 267 ion as the base peak by the direct elimination of OH from the M^+ ion peak (Scheme-6). The ion (m/e 116, RM 60.0) is formed by the direct loss of $C_7H_5NO_3$ from the base peak ion; the ion (m/e 106, RM 55.7) is formed by the direct loss of C_4H_7N from the base peak ion.

Piperidine compound shows m/e 281 ion as the base peak by the direct elimination of OH from the M^+ ion peak (m/e 314, RM 17.1, Scheme-7). The ion (m/e 198, RM 7.1) is formed by the direct loss of C_5H_9N from the base peak ion; the ion (m/e 130, RM 62.9) is formed by loss of $C_7H_5NO_3$ from the base peak ion.

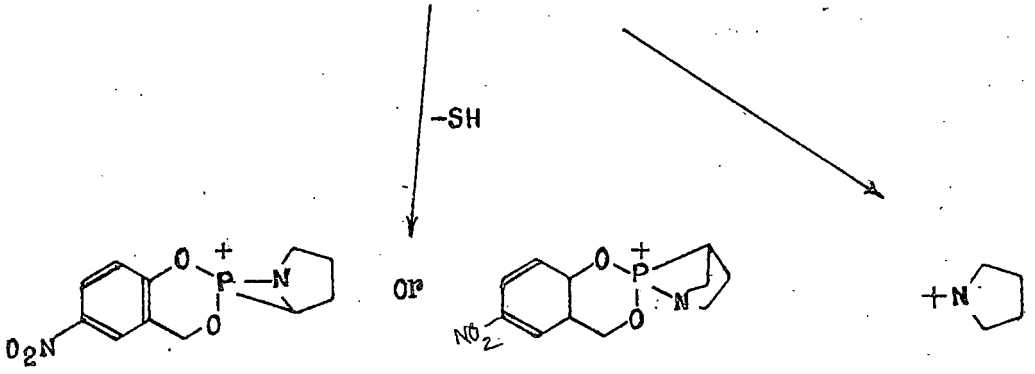
Benzylamide compound shows m/e 339 ion as the base peak by the direct elimination of OH from the parent molecular ion (m/e 372, RM 21.4, Scheme-8). The ion (m/e 259, RM 15.7) is formed by the direct loss of C_8H_{17} from the M^+ ion peak, the ion (m/e 198, RM 20) is formed by the loss of $C_9H_{19}N$ from the base peak ion. The ion (m/e 230, RM 7.1) is formed by the loss of CH_3N from the ion m/e 259.



Scheme 6 Mass Fragmentation of BD_{14}.

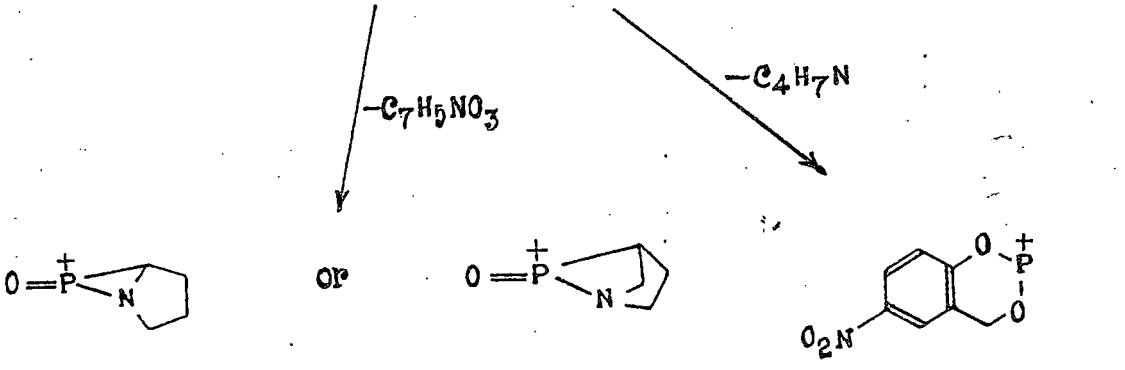


m/e 300 (M^+, 21.4%)



m/e 267 (base peak)

m/e 70 (35·7%)

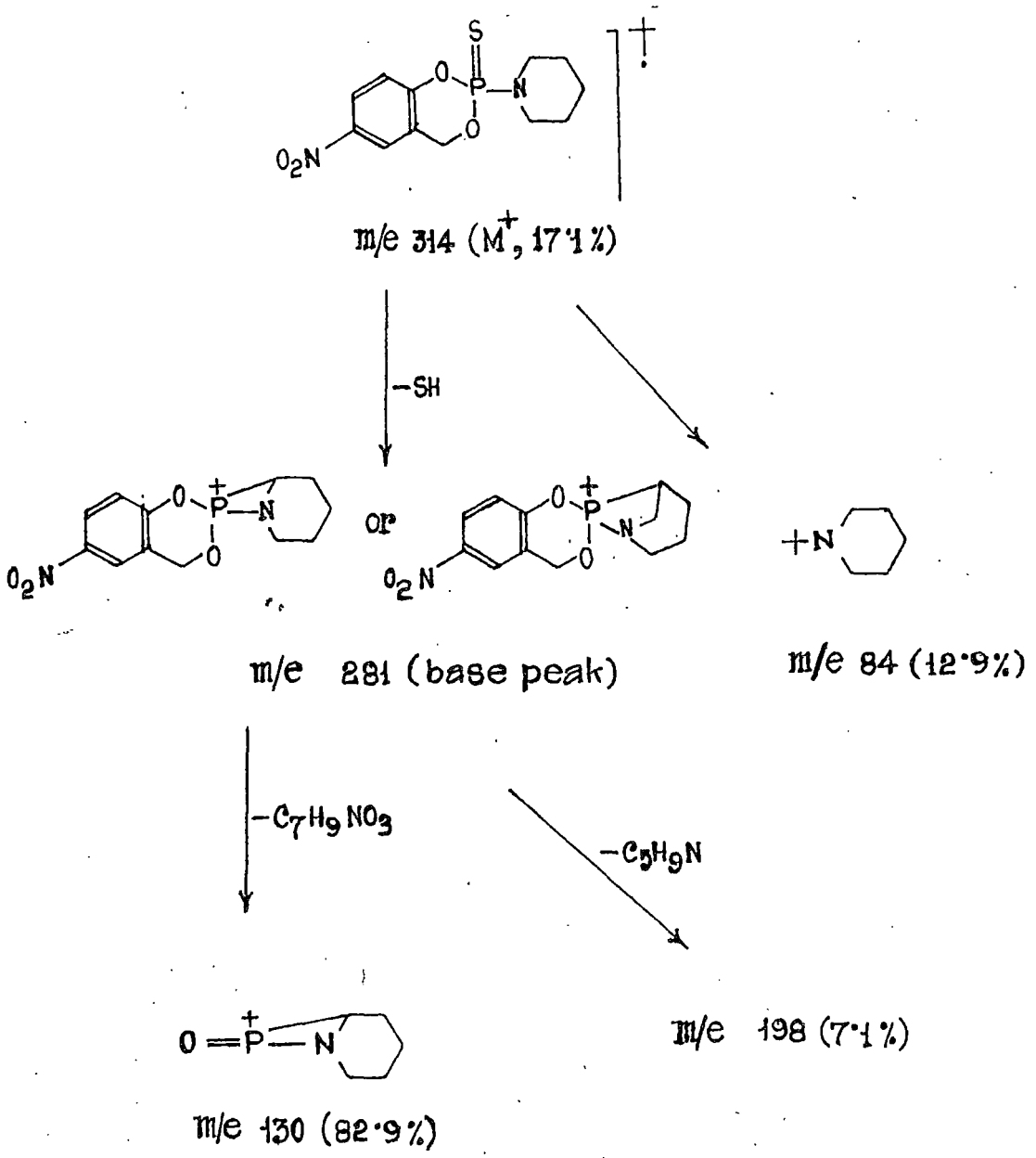


m/e 116 (80·0%)

m/e 198 (25·7%)

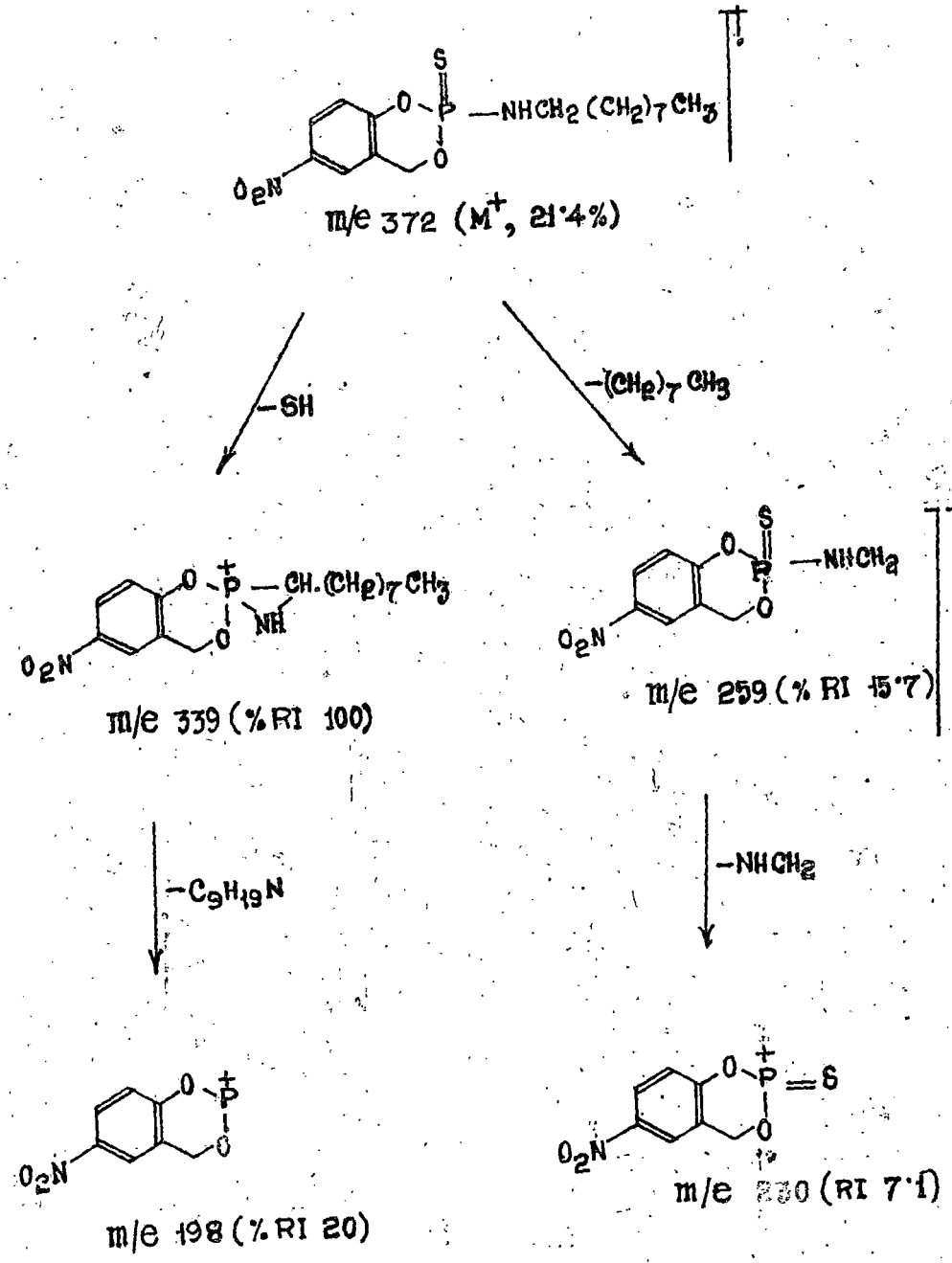
Scheme

Mass fragmentation processes in BD-15



Scheme – 7

Mass fragmentation of BD-16

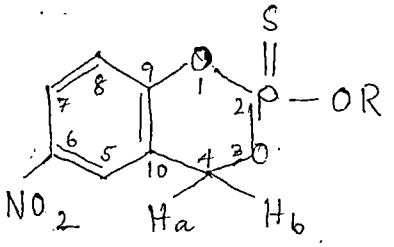


Scheme – 8

Mass fragmentation of BD-17

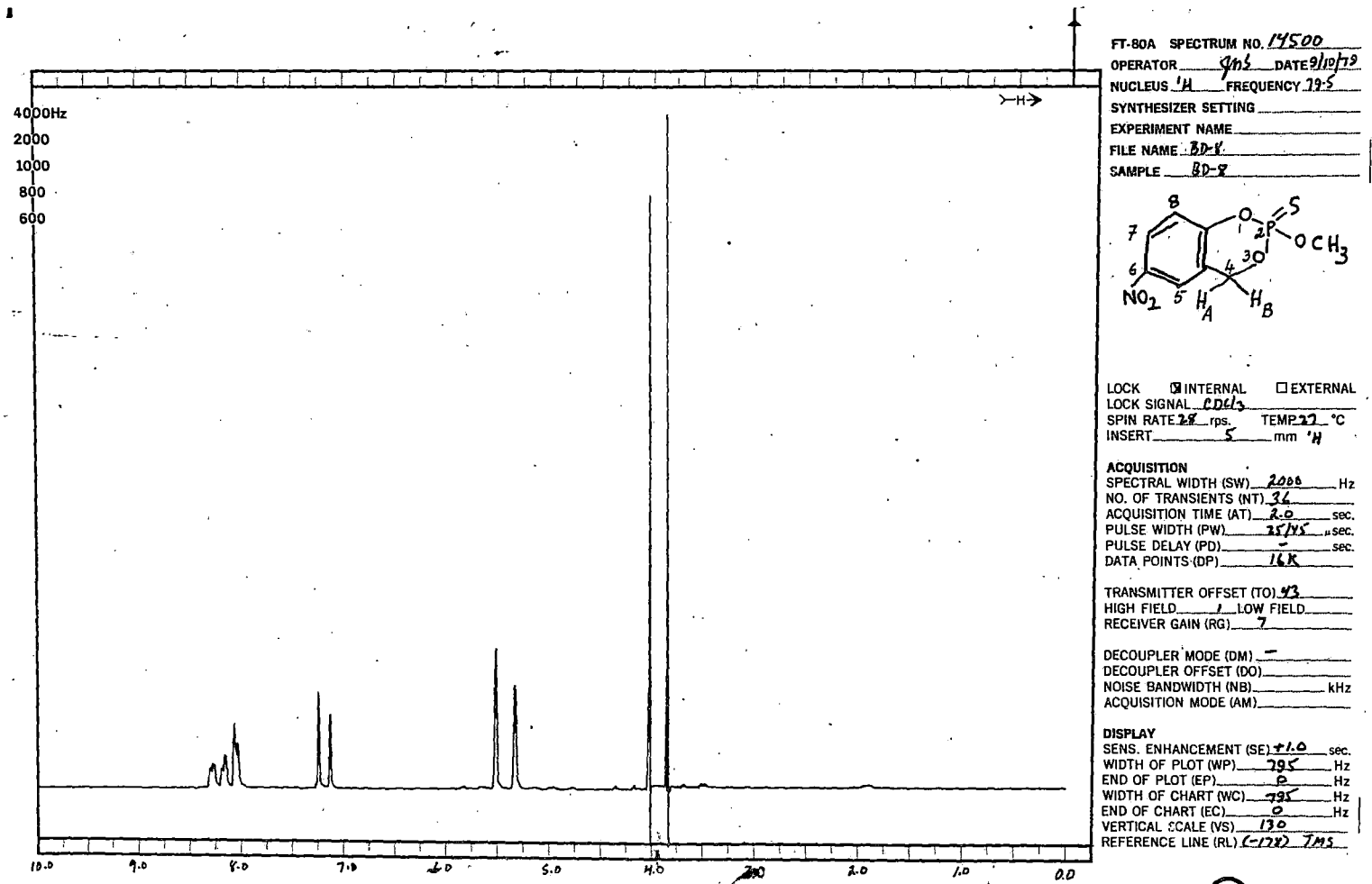
6. DISCUSSION ON NMR SPECTRA:

The ^1H NMR spectra of several 2-alkoxy/phenoxy-6-nitro-4H-1,3,2 benzodioxaphosphorin-2-sulphide have been reported by Das et al[21].



R = CH_3 (BD-8)
= C_2H_5 (BD-4)
= n-C_3H_7 (BD-3)
= n-C_4H_9 (BD-6)
= i-C_3H_7 (BD-5)
= i-C_4H_9 (BD-7)
= $C_2H_4OCH_3$ (BD-1)
= $C_2H_4OC_2H_5$ (BD-2)
= C_6H_5 (BD-9)

The ^1H NMR signal at δ =5.4 ppm in BD-1, BD-2, BD-9 and BD-10 (and also in other alkylamidophosphorothionates) is different from that of other compounds. Fig. 34 is the ^1H NMR spectrum of BD-8, Fig. 35 is the ^1H NMR spectrum of BD-9 and Fig. 7 is that of BD-10. If we study the three charts (Figs. 34,35 & 7) in the given order, it is fairly evident that the chemical shift difference of the protons H_{4A} & H_{4B} is increasing in going from BD-8 to BD-10. Also, the 2-substituent is at the sametime increasing in bulk, and probably spending more time in the conformation with the least steric interactions. In BD-10 (also in other alkylamidophosphorothionates), this may be mainly in one conformation; while in BD-8



FT-80A SPECTRUM NO. 14500
OPERATOR GNS DATE 9/10/79
NUCLEUS ^1H FREQUENCY 79.5
SYNTHESIZER SETTING
EXPERIMENT NAME
FILE NAME BD-8
SAMPLE BD-8

LOCK ☒ INTERNAL ☐ EXTERNAL
LOCK SIGNAL $CDCl_3$
SPIN RATE 28 rps. TEMP 27 °C
INSERT 5 mm ^1H

ACQUISITION
SPECTRAL WIDTH (SW) 2000 Hz
NO. OF TRANSIENTS (NT) 32
ACQUISITION TIME (AT) 2.0 sec.
PULSE WIDTH (PW) 25/45 μsec.
PULSE DELAY (PD) – sec.
DATA POINTS (DP) 16K

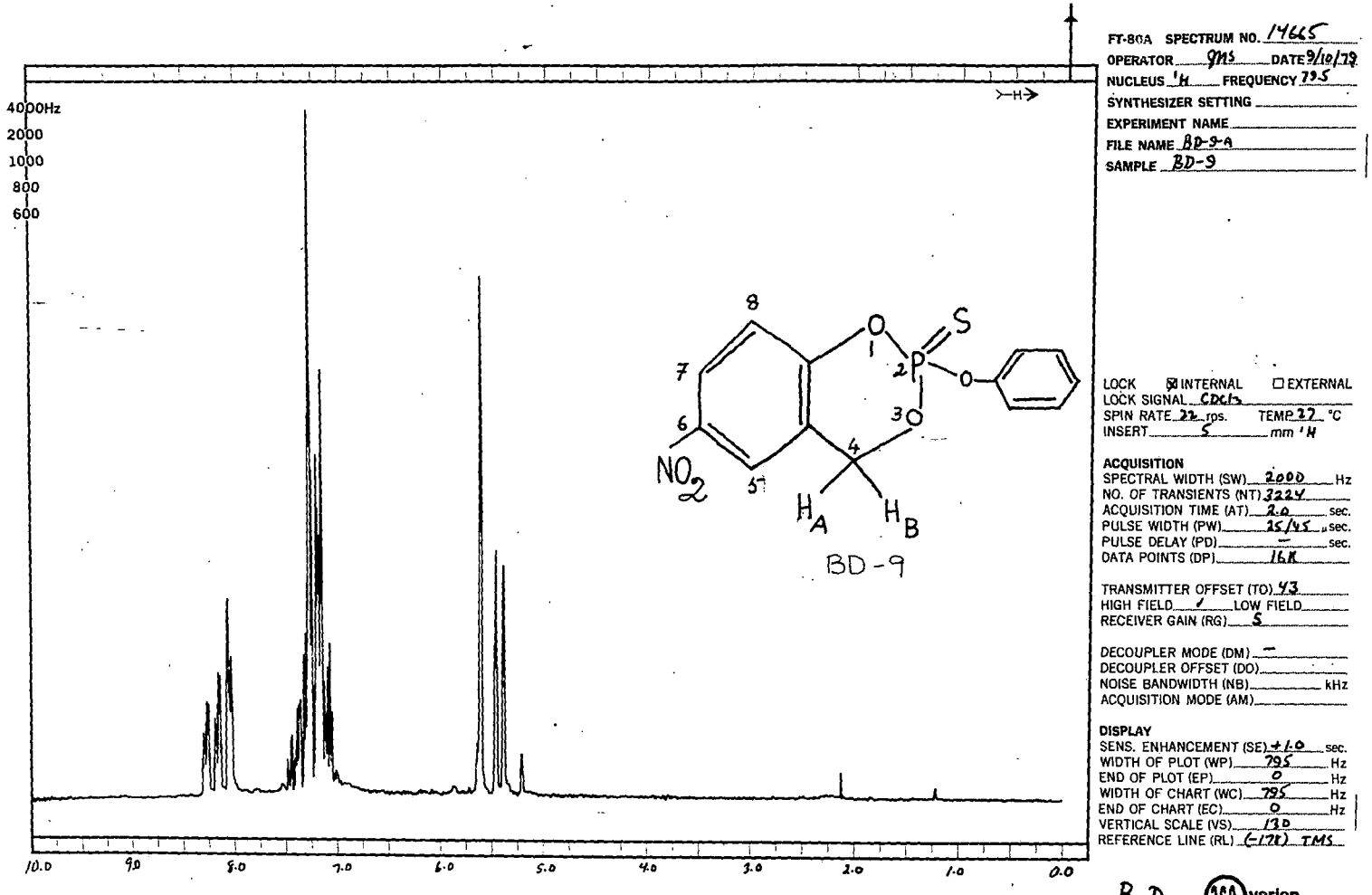
TRANSMITTER OFFSET (TO) 43
HIGH FIELD / LOW FIELD
RECEIVER GAIN (RG) 7

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

DISPLAY
SENS. ENHANCEMENT (SE) +1.0 sec.
WIDTH OF PLOT (WP) 795 Hz
END OF PLOT (EP) 0 Hz
WIDTH OF CHART (WC) 795 Hz
END OF CHART (EC) 0 Hz
VERTICAL SCALE (VS) 130
REFERENCE LINE (RL) (-172) TMS

B. Das varian PRINTED IN U.S.A.

Fig. 34. ^1H NMR spectrum of 2-Methoxy-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-sulphide.



FT-80A SPECTRUM NO. 14665
OPERATOR gns DATE 9/10/79
NUCLEUS ^1H FREQUENCY 79.5
SYNTHESIZER SETTING
EXPERIMENT NAME
FILE NAME BD-9-A
SAMPLE BD-9

LOCK ☒ INTERNAL ☐ EXTERNAL
LOCK SIGNAL CDCl3
SPIN RATE 22 rps. TEMP 27 °C
INSERT 5 mm ^1H

ACQUISITION
SPECTRAL WIDTH (SW) 2000 Hz
NO. OF TRANSIENTS (NT) 3224
ACQUISITION TIME (AT) 2.0 sec.
PULSE WIDTH (PW) 25/45 μsec.
PULSE DELAY (PD) — sec.
DATA POINTS (DP) 16K

TRANSMITTER OFFSET (TO) 43
HIGH FIELD / LOW FIELD
RECEIVER GAIN (RG) 5

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

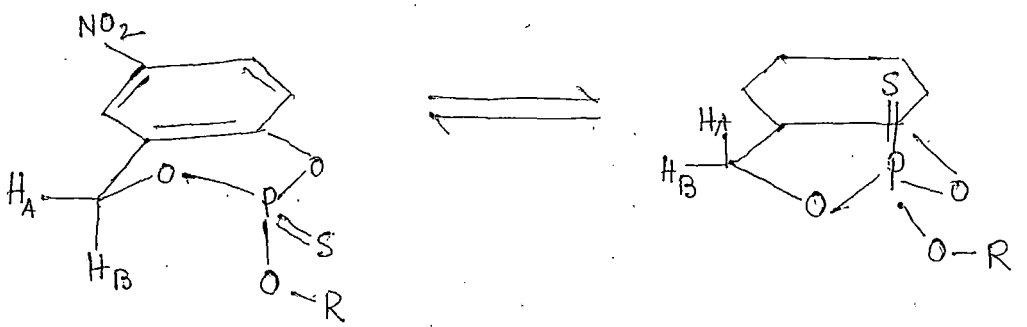
DISPLAY
SENS. ENHANCEMENT (SE) +1.0 sec.
WIDTH OF PLOT (WP) 795 Hz
END OF PLOT (EP) 0 Hz
WIDTH OF CHART (WC) 795 Hz
END OF CHART (EC) 0 Hz
VERTICAL SCALE (VS) 130
REFERENCE LINE (RL) (-172) TMS

B. Das varian PRINTED IN U.S.A.

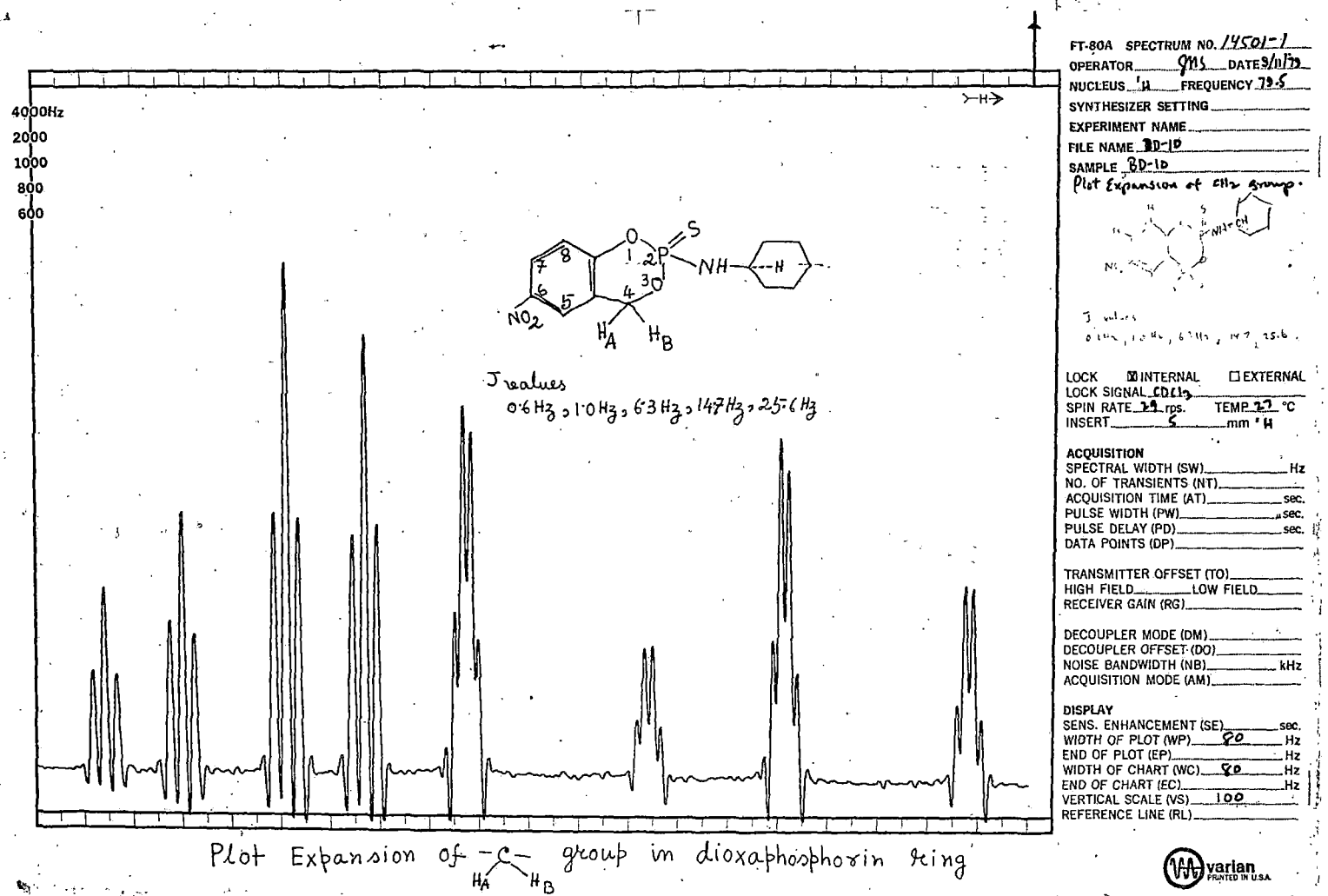
Fig. 35. ^1H NMR spectrum of 2-Phenoxy-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-sulphide.

(also in BD-4, BD-3, BD-6 etc.) it appears that both the chemical shift difference and the difference between $^{3}J_{P\text{-}O\text{-}C\text{-}H_{4A}}$ and $^{3}J_{P\text{-}O\text{-}C\text{-}H_{4B}}$ are quite small. This suggests that the molecule exists as an average of the two conformations with rapid interconversion between them. In the case of BD-9, it seems that an intermediate situation prevails, similar to BD-1 and BD-2.

In either of the conformations I & II



one of the couplings, $^{3}J_{P\text{-}O\text{-}C\text{-}H_{4A}}$ or $^{3}J_{P\text{-}O\text{-}C\text{-}H_{4B}}$ will be large and other one will be small, since the $P\text{-}O\text{-}C\text{-}H_4$ 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 in BD-10 (Fig. 36) shows couplings of 0.6, 1.0, 6.3, 14.7 and 25.6 Hz. Since the 14.7 Hz coupling is certainly the geminal $^{2}J_{H_A\text{-}H_B}$ coupling constant, this then leaves 6.3 and 25.6 Hz as the two $^{3}J_{P\text{-}O\text{-}C\text{-}H}$ coupling



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

Fig. 36. Plot expansion of the $-CH_2-$ group (in BD-10), 5.0 – 5.8 ppm region.

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, similar to BD-1 and BD-2.

The small couplings seen in Fig. 36 arise from coupling to the aromatic ring protons H_5, H_7 and H_8. The conformation 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 180^0 belongs to H_B, and we can identify it as the upfield group of lines showing equal coupling of 0.6 Hz to the three aromatic protons H_5, H_7 and H_8. Proton H_A is more strongly coupled to H_5 and H_7 with $J = 1.0$ Hz, but not to H_8. This can be confirmed by spin-decoupling experiments shown in Fig. 37 and Fig. 38. In Fig. 37, aromatic protons H_5 and H_7 were irradiated at 8.1 ppm and the triplet structure of H_A disappeared; the quartet structure of H_B also collapsed due to irradiation of H_5 and H_7, which removed the effects of their spins, but the residual line width masks the remaining small coupling to H_8. However, in Fig. 38, in which only H_8 was irradiated, we can see that the quartets have become triplets as expected.

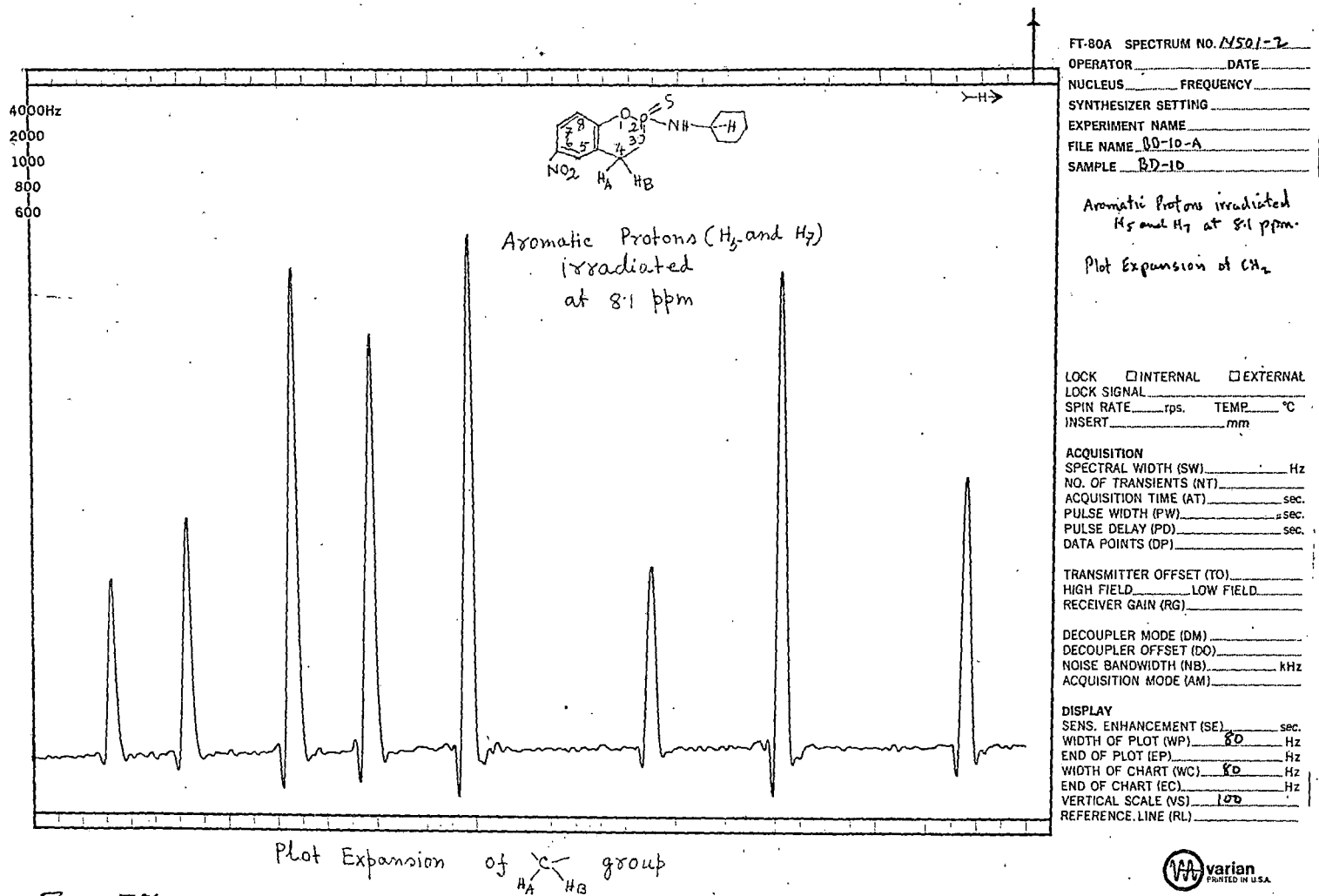


Fig. 37. Plot expansion of $-CH_2-$ group in BD-10 (aromatic protons H_5 and H_7 irradiated at 8·1 ppm)

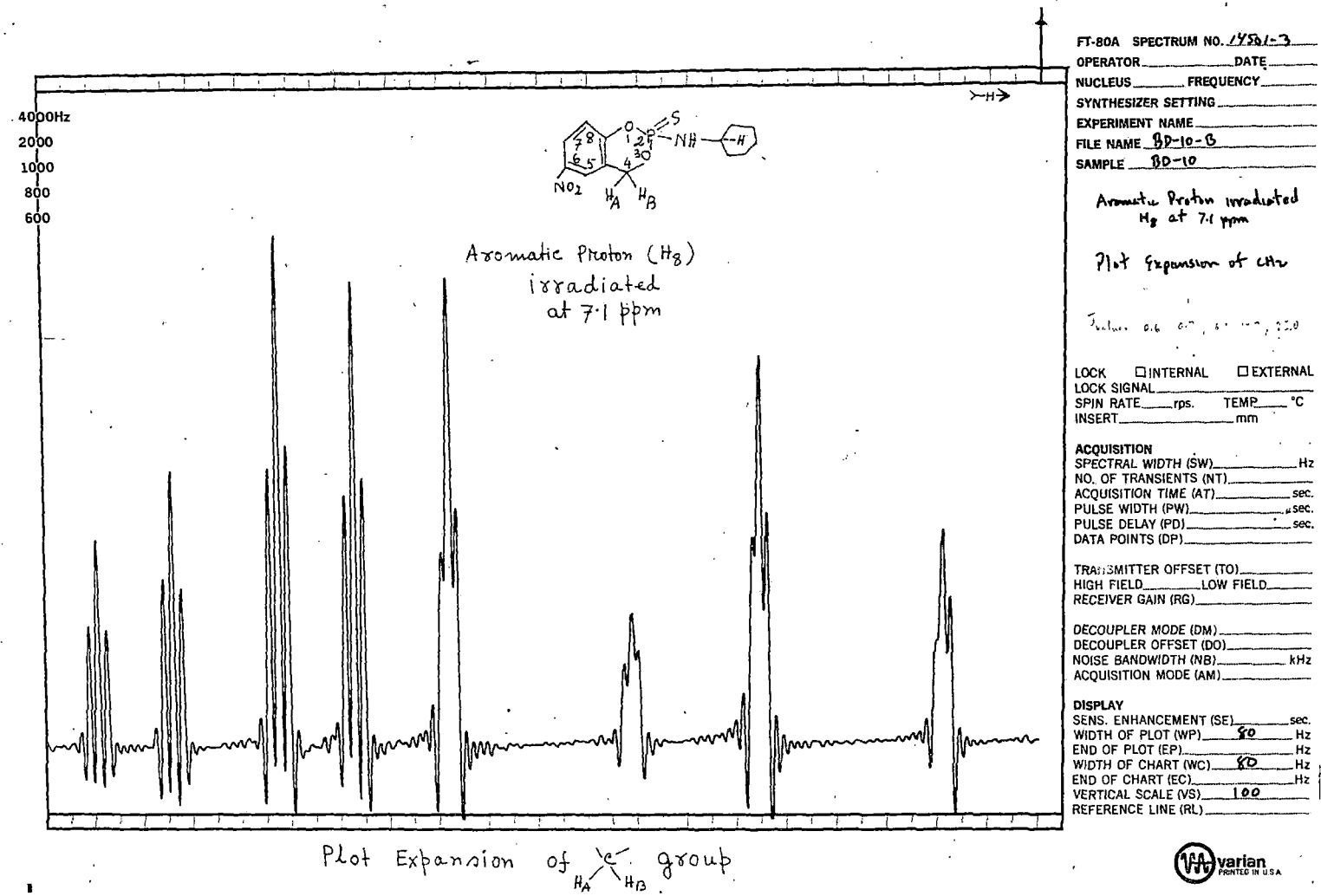
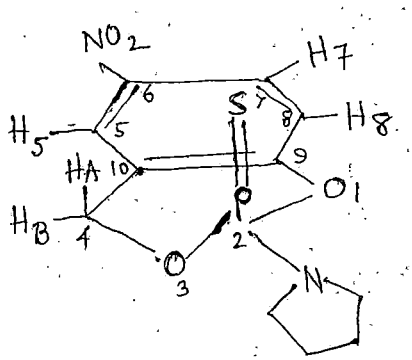
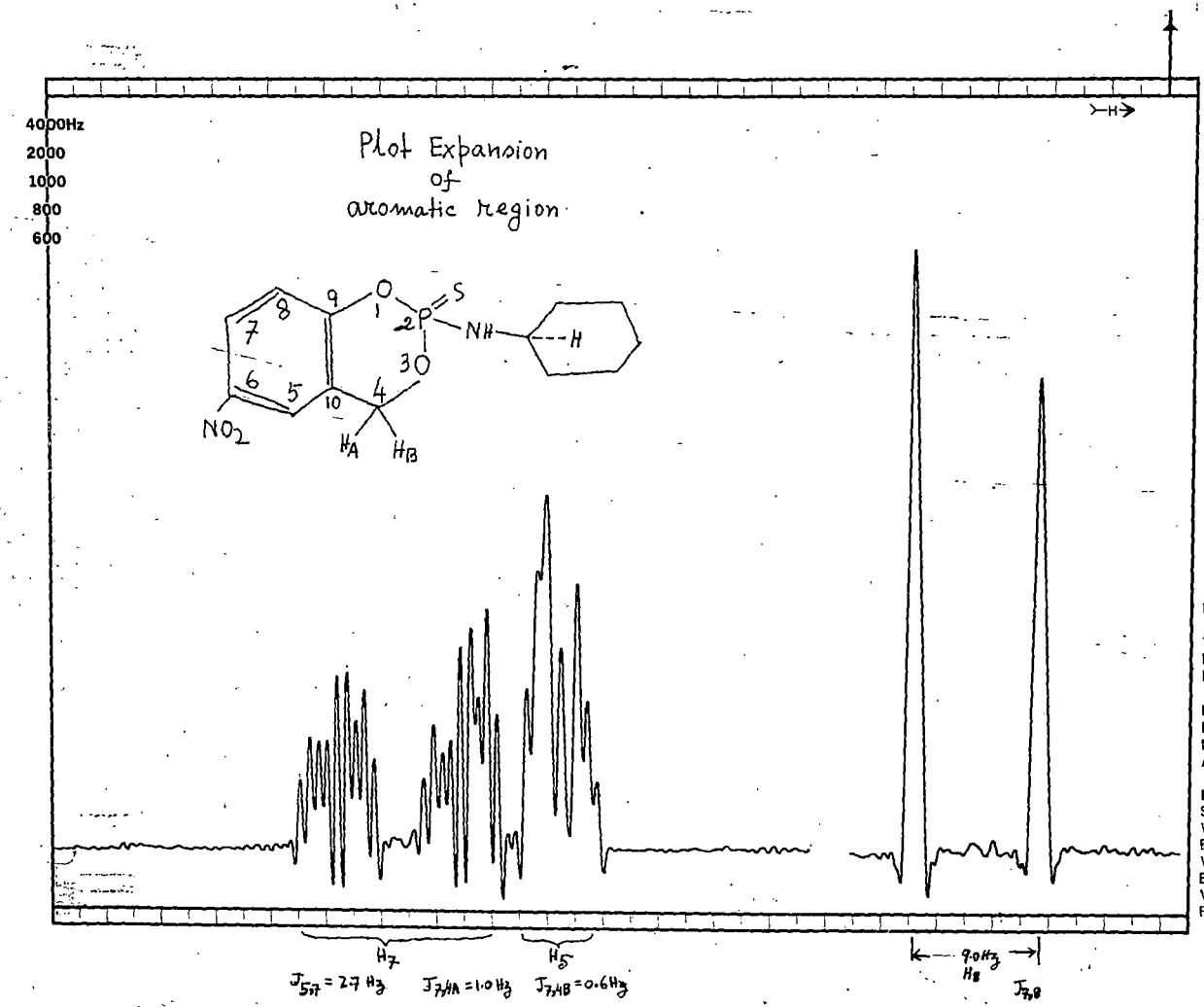


Fig. 38. Plot expansion of $-CH_2-$ group in BD-10 (aromatic proton H_8 irradiated at 7.1 ppm)

The plot expansion of the aromatic region (Fig. 39 for BD-10) shows the basic ABX pattern with the additional long range couplings to H_5 and H_7. The interpretation of this spectral region is facilitated by examining Fig. 40 in which the $-CH_2-$ group in the dioxaphosphorin ring was decoupled. In Fig. 40, H_7 appears as an eight line pattern. In Fig. 39, each line of this pattern is split by the protons H_{4A} and H_{4B} into triplets with 0.8 Hz spacing. This is the average of the actual couplings ($J_{H_7 - H_{4A}} = 1.0$ Hz ; $J_{H_7 - H_{4B}} = 0.6$ Hz), due to the fact that H_A and H_B are strongly coupled ($^2J_{H_A - H_B} = 14.7$ Hz) and separated by a small chemical shift.

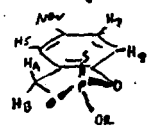
Fig. 41 and Fig. 42 are the plot expansions of BD-15 and BD-16 respectively. The $-CH_2-$ protons in the dioxaphosphorin ring are non-equivalent and have chemical shifts in both compounds of 5.2 ppm and 5.65 ppm. The coupling to the ^{31}P nucleus is 25 Hz for one the $-CH_2-$ protons and 6.6 Hz for the other. A Dreiding model of the molecules seems to have a stable conformation in which proton H_B is quasi-equatorial and proton H_A is quasi-axial.





FT-80A SPECTRUM NO. 14501-4
OPERATOR _____ DATE _____
NUCLEUS _____ FREQUENCY _____
SYNTHESIZER SETTING _____
EXPERIMENT NAME _____
FILE NAME _____
SAMPLE BD-10

Plot Expansion of aromatic region.



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

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

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

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

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

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Fig. 39. Plot expansion of the aromatic region of BD-10

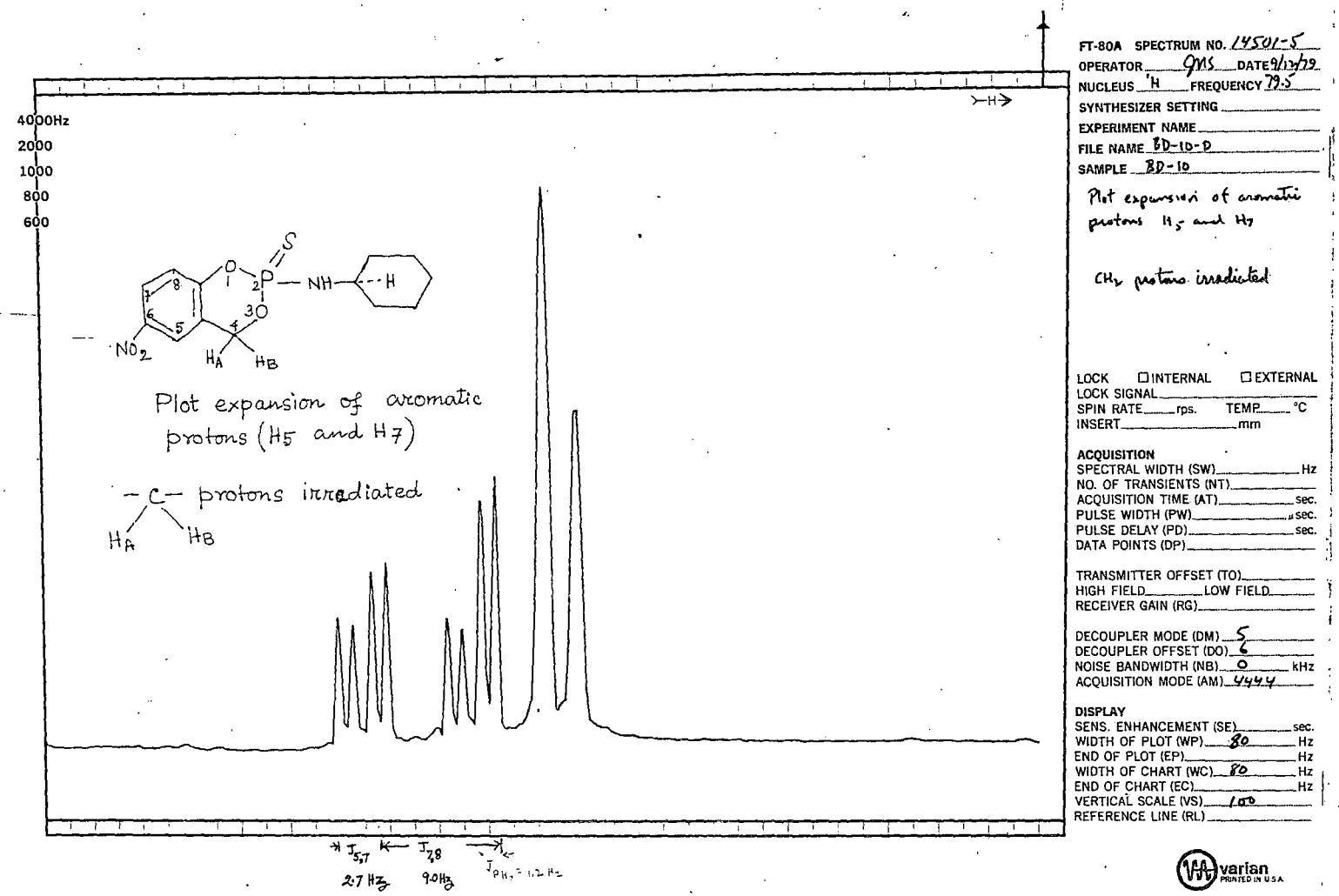
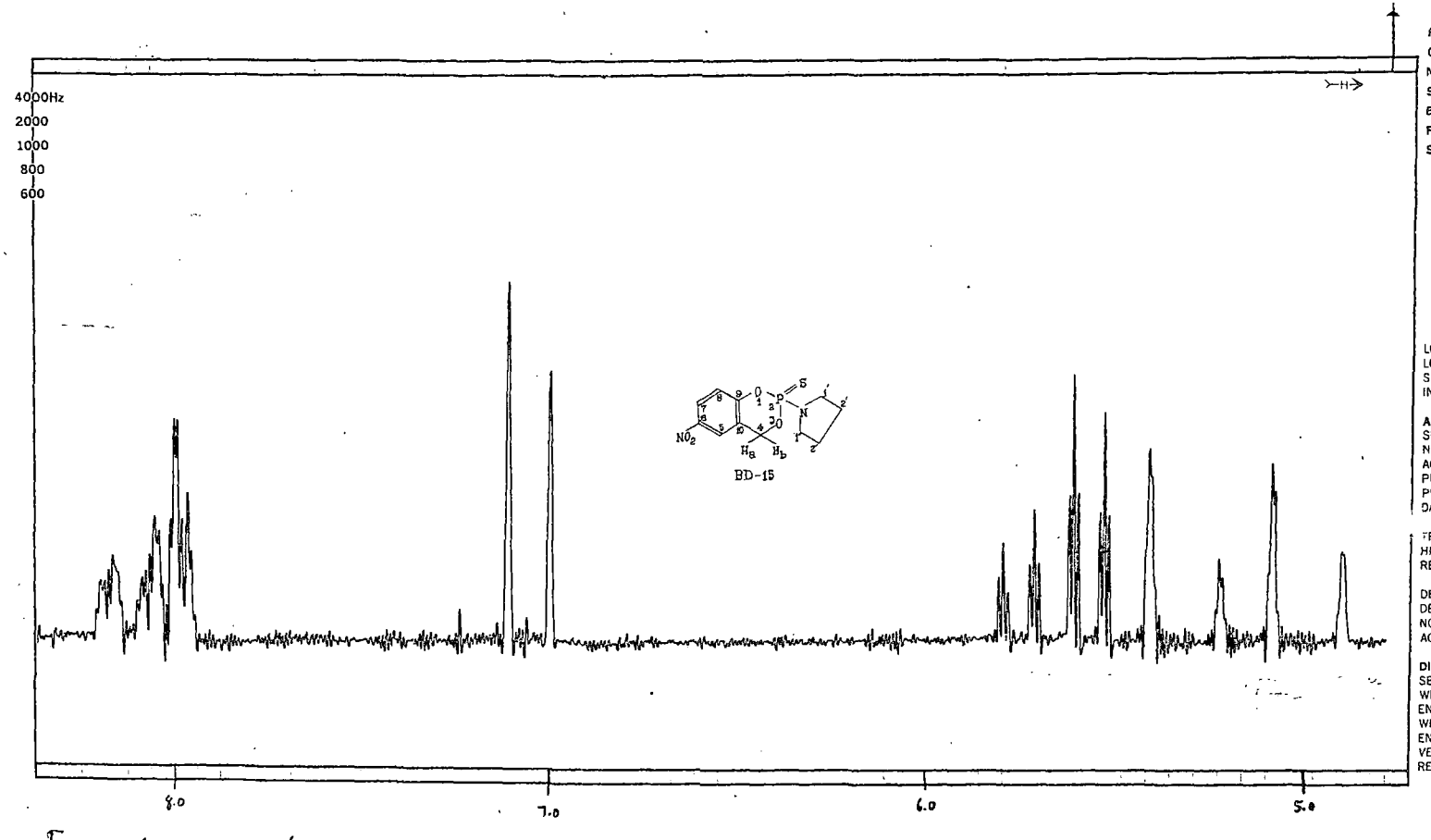


Fig. 40 : Plot expansion of the aromatic protons H_5 and H_7 in BD-10 with the $-CH_2-$ protons (in the dioxaphosphorin ring) irradiated.



FT-80A SPECTRUM NO. 14992-1
OPERATOR _____ DATE _____
NUCLEUS ^{1}H FREQUENCY _____
SYNTHESIZER SETTING _____
EXPERIMENT NAME _____
FILE NAME _____
SAMPLE BD-15

Plot Expansion
Scale: 1.0 Hz/Div.

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

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

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

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

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

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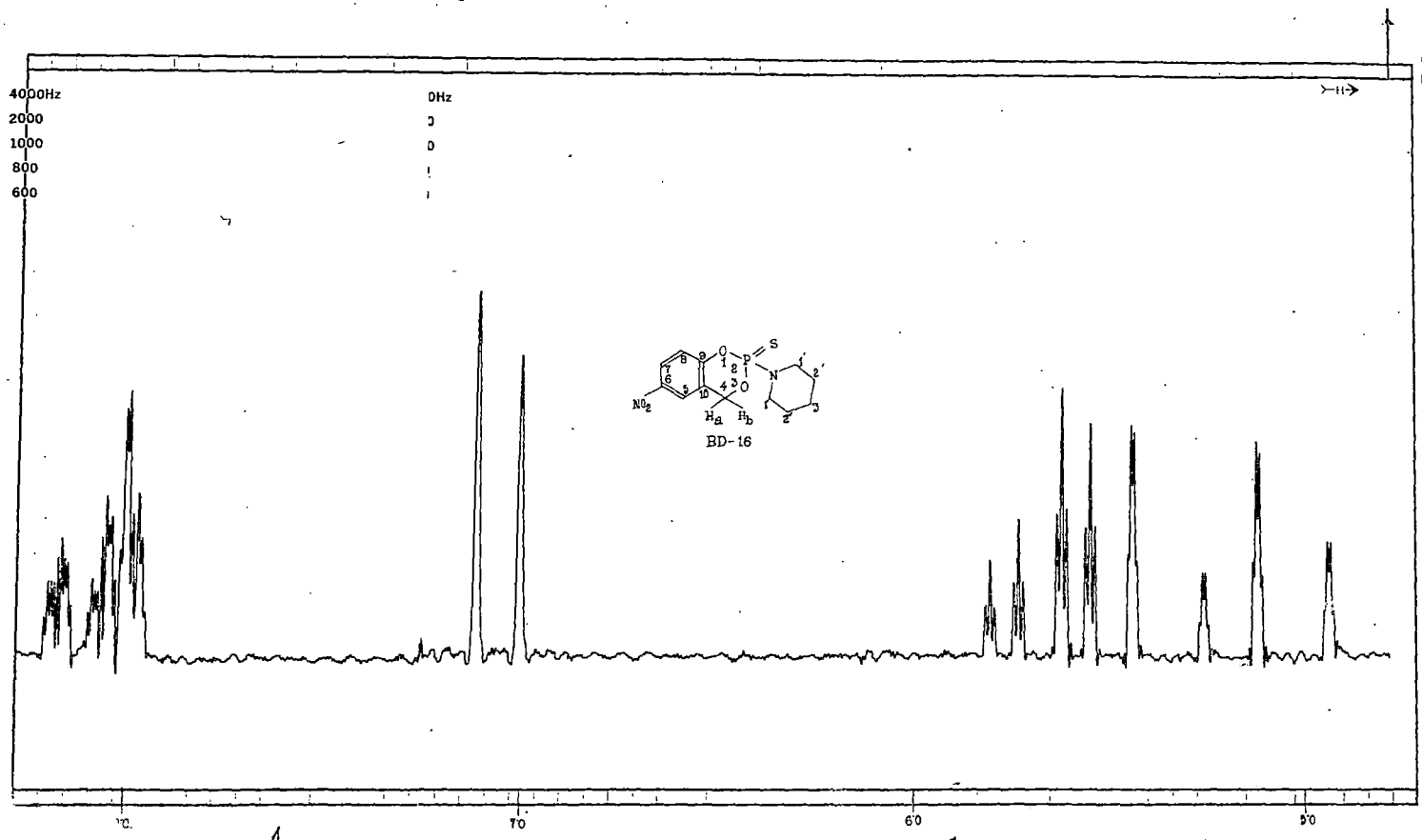
Fig. 41 ^{1}H NMR spectrum of BD-15 (Plot expansion)



BD-16

FT-80A SPECTRUM NO. 14993-1
OPERATOR _____ DATE _____
NUCLEUS ^1H FREQUENCY _____
SYNTHESIZER SETTING _____
EXPERIMENT NAME _____
FILE NAME _____
SAMPLE BD-16

Plot Expansion

Scale: 1.0Hz/div.

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

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

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

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

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

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Fig. 42: ^1H NMR spectrum of BD-16 (Plot expansion)

The assignment of the protons H_A and H_B in the spectrum is based on the coupling constants to the ^{31}P nucleus. The P-O-C-H dihedral angle is 180° for the quasi-equatorial proton H_B, and close to 60° for the quasi-axial proton H_A. Thus H_B is assigned to the 5.2 ppm proton ($^3J_{P-O-C-H_B}$ = 25 Hz) and H_A is assigned to the 5.65 ppm proton ($^3J_{P-O-C-H_A}$ = 6.5 Hz). This results in the quasi-equatorial proton having the smaller chemical shift, and the quasi-axial having the larger one, which is contrary to expectations based on aromatic ring current effects. However, the P = S bond also can exert the anisotropic magnetic effects and these might easily reverse the order of the chemical shifts. It is easier to live with this than with a violation of the Karplus-type relationship for spin-couplings which seems generally valid.

Proton H_8 is identified at 7.06 ppm by its 9 Hz coupling constant to proton H_7 at 8.14 ppm. This leaves proton H_5 at 8.0 ppm. Irradiation of H_5 and H_7 resulted in disappearance of the long range couplings which split H_B into quartets and H_A into triplets. Thus, H_B is coupled equally to all three aromatic protons with coupling constants of about 0.7 Hz, while H_A is coupled to two of the three aromatic protons with equal coupling constants of approximately 1.0 Hz. Since the quasi-axial and quasi-equatorial protons H_A and H_B interact differently with the sigma and pi electrons of the aromatic ring system, these differences in coupling are not surprising. In particular, the larger coupling for the quasi-axial proton seems in accord with the idea that

the bonding electron density for H_A is distributed along axes more or less perpendicular to the plane of the aromatic ring and that this allows more overlap with the ring pi-electron system whose maximum probablity function also lies above and below the plane of the ring.

Fig. 27a and Fig. 31a show the proton noise-decoupled ^{13}C spectra of BD-15 and BD-16 at 20.0 MHz. The saturated carbons 2 and 3 bonds from the phosphorus show splitting due to ^{13}C - ^{31}P spin coupling. These couplings are slightly different in BD-15 and BD-16. The coupling to the CH_2 carbon (C_4) in the dioxaphosphorin ring changes only from 5.53 Hz in BD-15 to 5.75 Hz in BD-16. This probably means that the conformation is almost the same, and this is in accord with the small difference in ^{13}C chemical shifts, 66.11 and 65.62 ppm. The change in $\delta(^{13}C)$ is only 0.5 ppm for this carbon (C_4), and since ^{13}C chemical shifts are often quite sensitive to conformation, it supports the similarity of the structures.

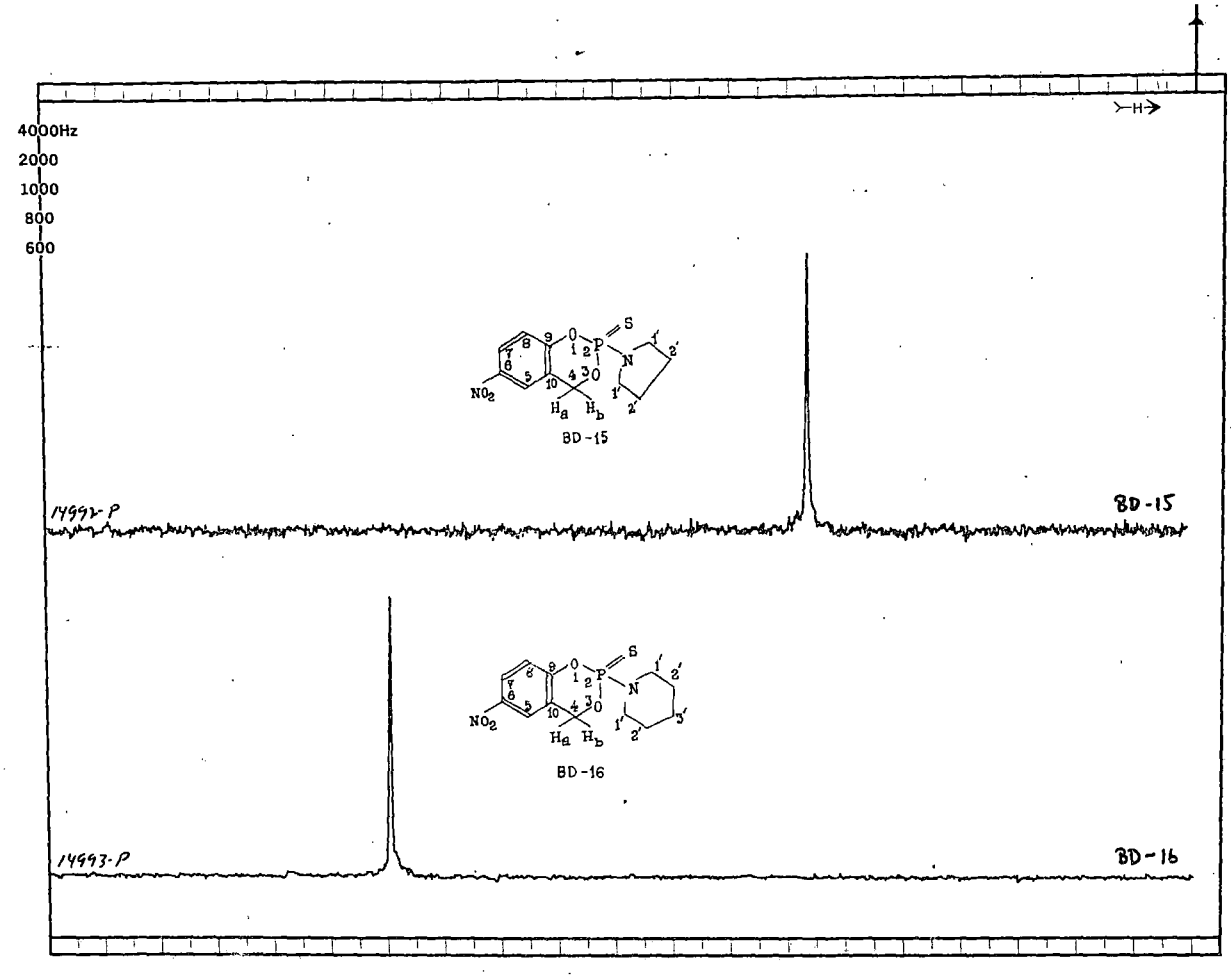
The CH_2's next to the nitrogen are coupled with J -values of 5.83 and 3.67 Hz respectively in BD-15 and BD-16. The CH_2's β to the nitrogen have J -values of 9.87 and 3.63 Hz. These large differences are due to the differences in the P-N-C bond angles for the 5- and 6- membered rings. Fig. 27b and Fig. 31b show the plot expansions of the aliphatic regions of ^{13}C spectrum in BD-15 and BD-16, clearly revealing the doubling of the lines due to the spin of the ^{31}P nucleus.

Fig. 27c is a plot expansion of the 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 constant (Hz)
C_8	8.19
C_{10}	12.38
C_9	7.30
C_7	0.93

The compound BD-16 shows smaller shifts for the carbons C_9 and C_{10} compared to those in BD-15 which again support a very minor difference in the conformations. Fig. 31b shows that the carbon γ to the ring nitrogen in BD-16 is coupled to the phosphorus with a 1.2 Hz coupling constant.

Fig. 43 is the 32.2 MHz ^1H broad-band decoupled ^{31}P NMR spectra of BD-15 and BD-16. Both compounds gave a sharp ^{31}P resonance line. There is a displacement of 3.63 ppm in the chemical shift for the phosphorus between the two compounds. Selective decoupling (Fig. 44, for BD-16) of the protons adjacent to the nitrogen (arrows) narrowed the otherwise broad line and showed that the ^{31}P is strongly coupled to protons H_A and H_B (attached to C_4). The couplings are reduced in this spectrum because the irradiation at 3.35 ppm partially collapses the couplings to the protons at 5.2 and 5.65 ppm. However, the ratio of the couplings is



14992-P

FT-80A SPECTRUM NO. 14993-P
OPERATOR JMS DATE 2/18/81
NUCLEUS ^{31}P FREQUENCY 32.203
SYNTHESIZER SETTING 29.951
EXPERIMENT NAME
FILE NAME
SAMPLE BD-16 / BD-15

^{31}P

Decoupled

Chemical shift difference is 3.63 ppm.

LOCK ☒ INTERNAL ☐ EXTERNAL
LOCK SIGNAL CDCl3
SPIN RATE 30 rps. TEMP 30 °C
INSERT 5 mm

ACQUISITION
SPECTRAL WIDTH (SW) 1000 Hz
NO. OF TRANSIENTS (NT) 1
ACQUISITION TIME (AT) 4.0 sec.
PULSE WIDTH (PW) 4 μsec.
PULSE DELAY (PD) - sec.
DATA POINTS (DP) 16K

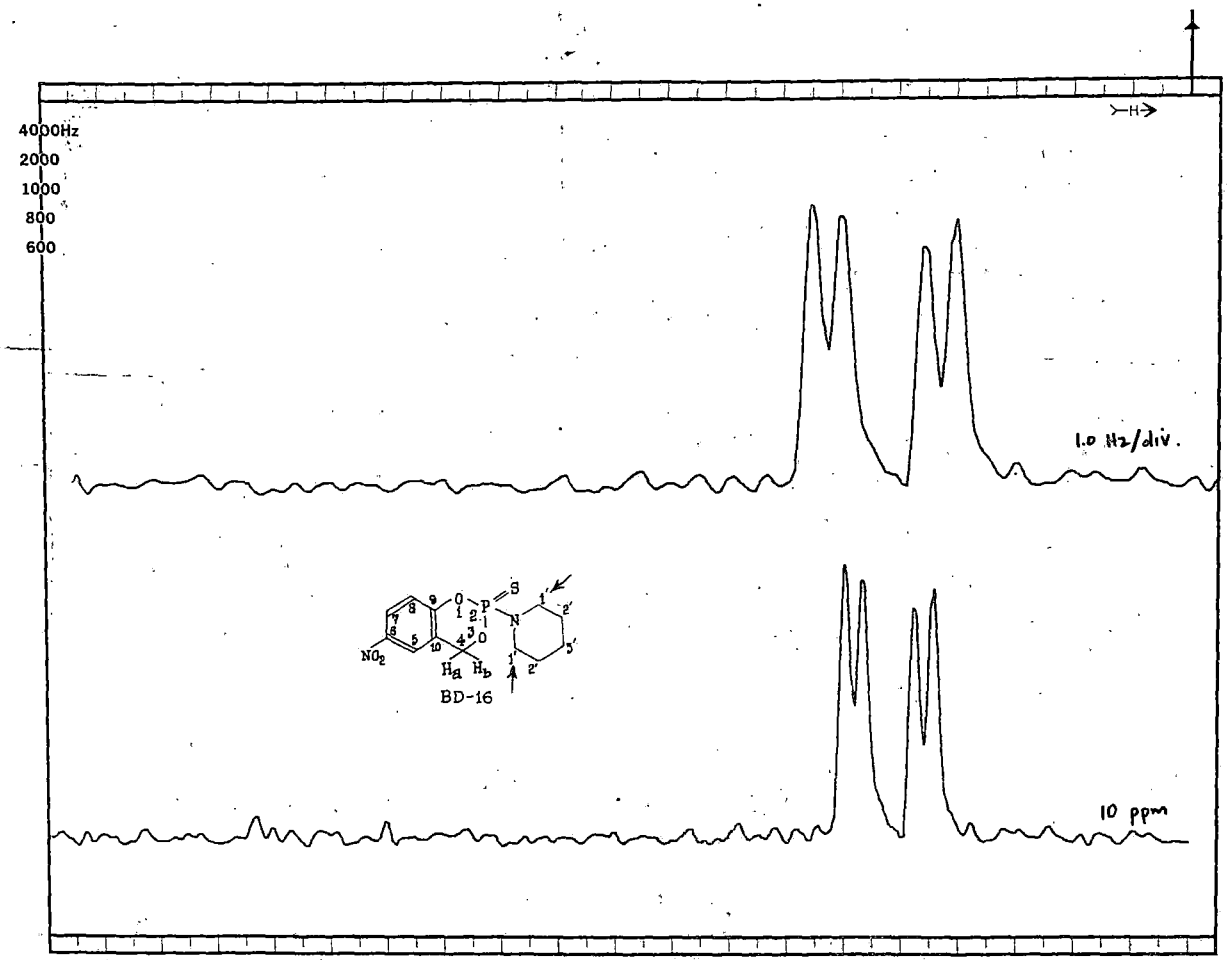
TRANSMITTER OFFSET (TO) 29.951
HIGH FIELD ✓ LOW FIELD
RECEIVER GAIN (RG) 8

DECOUPLER MODE (DM) 1
DECOUPLER OFFSET (DO) 50
NOISE BANDWIDTH (NB) -7 kHz
ACQUISITION MODE (AM) 0

DISPLAY
SENS. ENHANCEMENT (SE) 0.0 sec.
WIDTH OF PLOT (WP) 1000 Hz
END OF PLOT (EP) 0 Hz
WIDTH OF CHART (WC) 1000 Hz
END OF CHART (EC) 0 Hz
VERTICAL SCALE (VS) 100
REFERENCE LINE (RL) —

varian PRINTED IN U.S.A.

Fig. 43: ^{31}P NMR spectrum of BD-15 and BD-16 (^{1}H broad-band decoupled, chemical shift difference is 3.63 ppm)



FT-80A SPECTRUM NO. 4993-P-1
OPERATOR gms DATE 2/4/81
NUCLEUS ^{31}P FREQUENCY 32.203
SYNTHESIZER SETTING 29.951
EXPERIMENT NAME
FILE NAME
SAMPLE BD-16

^{31}P
Selective decoupling

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

ACQUISITION
SPECTRAL WIDTH (SW) 1000 Hz
NO. OF TRANSIENTS (NT)
ACQUISITION TIME (AT) sec.
PULSE WIDTH (PW) μsec.
PULSE DELAY (PD) sec.
DATA POINTS (DP)

TRANSMITTER OFFSET (TO) 29.951
HIGH FIELD / LOW FIELD
RECEIVER GAIN (RG) 8

DECOUPLER MODE (DM) 5
DECOUPLER OFFSET (DO) 6 (4280)
NOISE BANDWIDTH (NB) 0 kHz
ACQUISITION MODE (AM) 0

DISPLAY
SENS. ENHANCEMENT (SE) +0.5 sec.
WIDTH OF PLOT (WP) 32K/20K Hz
END OF PLOT (EP) Hz
WIDTH OF CHART (WC) Hz
END OF CHART (EC) Hz
VERTICAL SCALE (VS) 50/50
REFERENCE LINE (RL) —

varian PRINTED IN U.S.A.

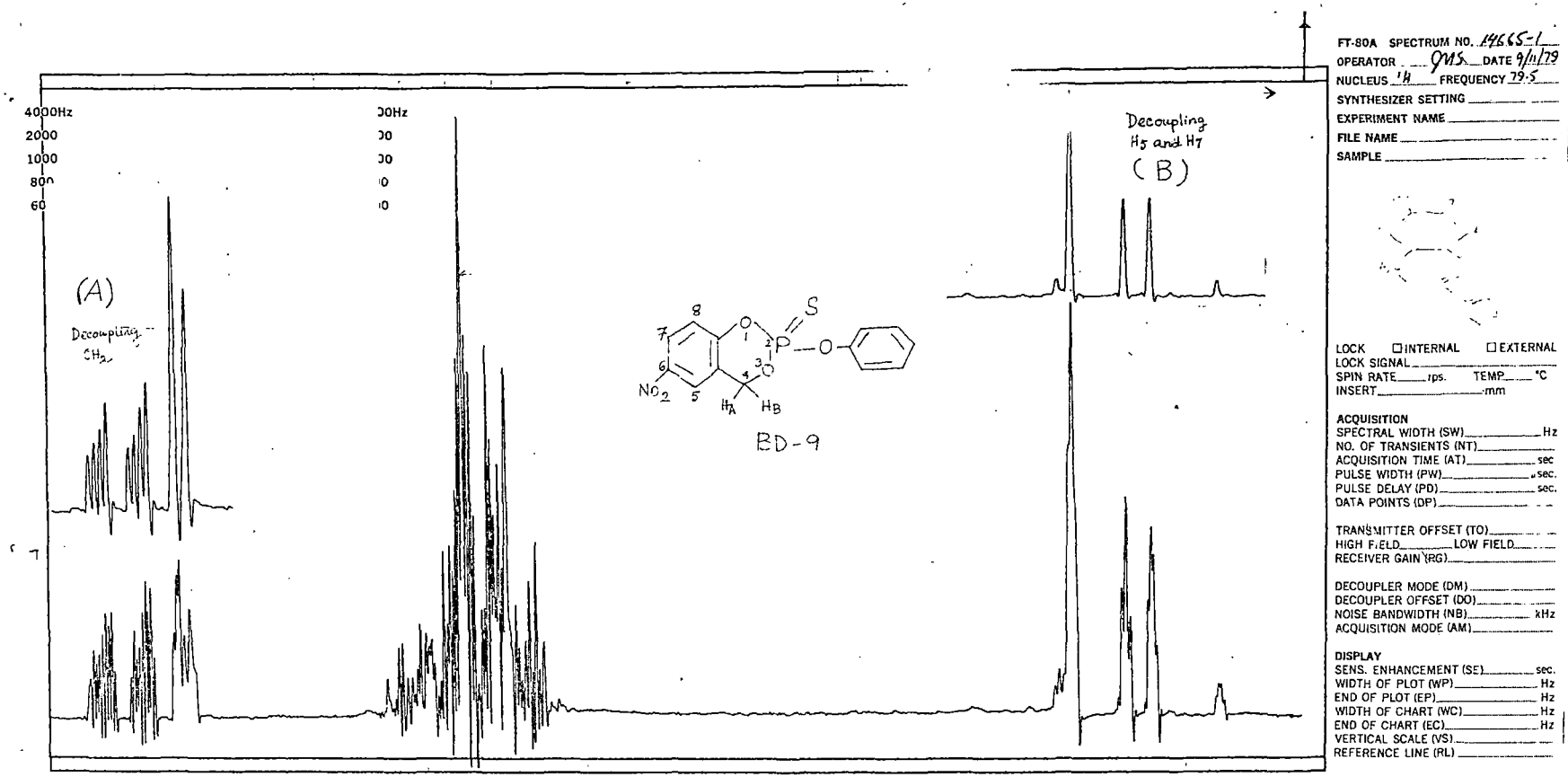
Fig. 44.: ^{31}P NMR spectrum of BD-16 (Selective decoupling)

still the same which is about 4:1. Selective decoupling of the protons for other compounds has not yet been performed.

Fig. 45 is a plot expansion of BD-9 [45(A) is the spectrum of the aromatic protons decoupled with $-CH_2-$ group, 45(B) is the spectral region of $-CH_2-$ group decoupled with H_5 and H_7; this spectrum (Fig. 45) shows that the same coupling patterns prevail in this molecule as found in BD-10, BD-15 and BD-16. The smaller separation of the lines in the CH_2 pattern of BD-9 compared to BD-10 indicates that the quasi-equatorial position of the 2-phenoxy substituent is only slightly favoured over the inverted form.

Fig. 46 shows that BD-8 also displays the long range couplings to the aromatic protons (Fig. 47 shows the CH_2 group and the $O-CH_3$). By irradiating the aromatic protons H_5 and H_7, shown in Fig. 48, the CH_2 group can be seen to be the AB part of the ABX pattern that arises from the CH_2 and the phosphorus. The geminal AB coupling is 14.5 Hz. The couplings $^3J_{P-O-C-H_A}$ and $^3J_{P-O-C-H_B}$ must be quite small. This is consistent with nearly equal populations of the two conformations.

The temperature dependant ^1H NMR spectra at 270 MHz in the temperature range -70^oC to $+50^oC$ (203^oK to 323^oK) of the methoxy compound (BD-8) have been given in Fig. 49 —74 . It is obvious from the charts that as the temperature is varied, the rates of



Scale : 1.0 Hz/division

Fig. 45. Plot expansion of BD-9 [45(A): aromatic protons decoupled with $-CH_2-$ group; 45(B): CH_2 group region decoupled with H_5 and H_7]

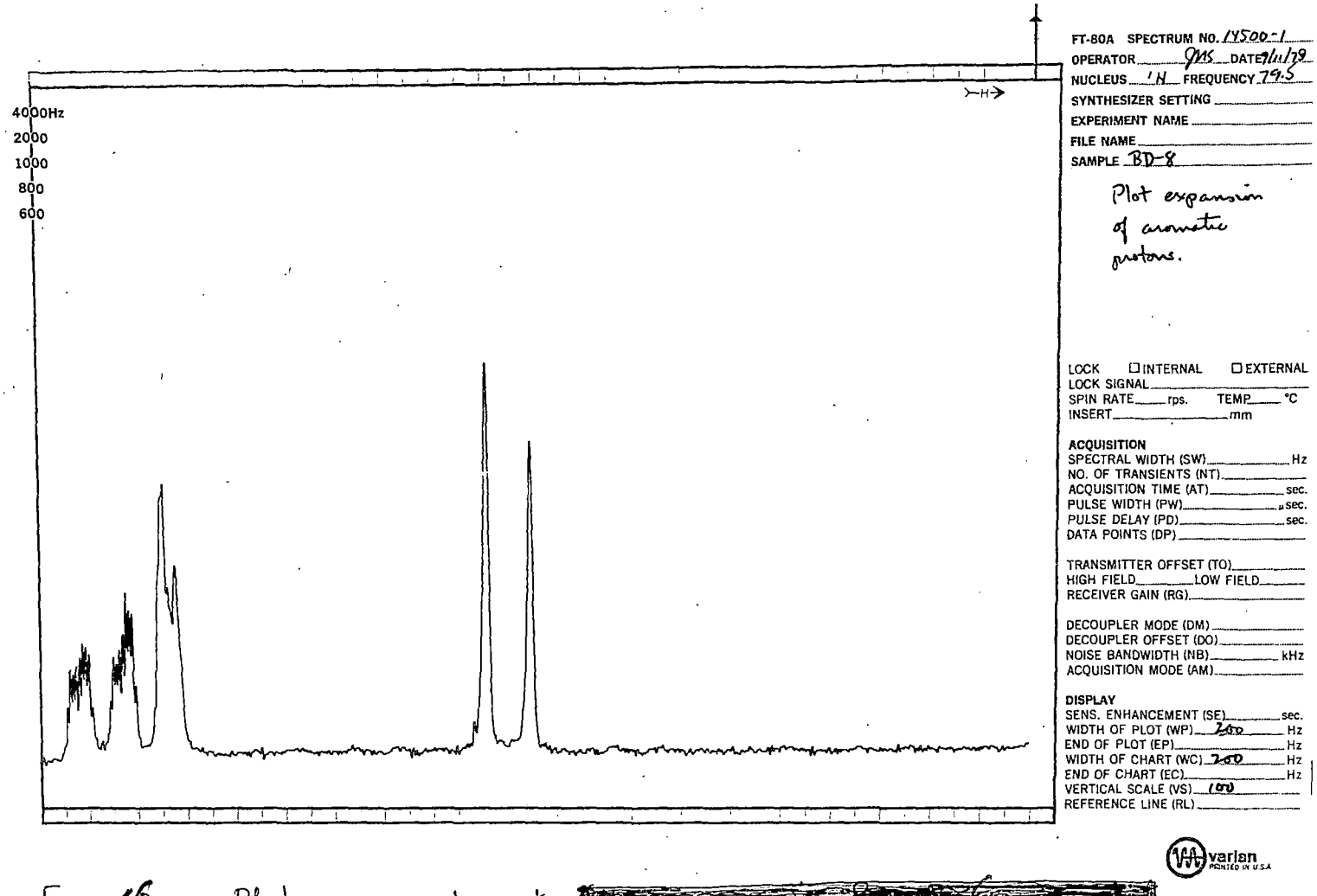
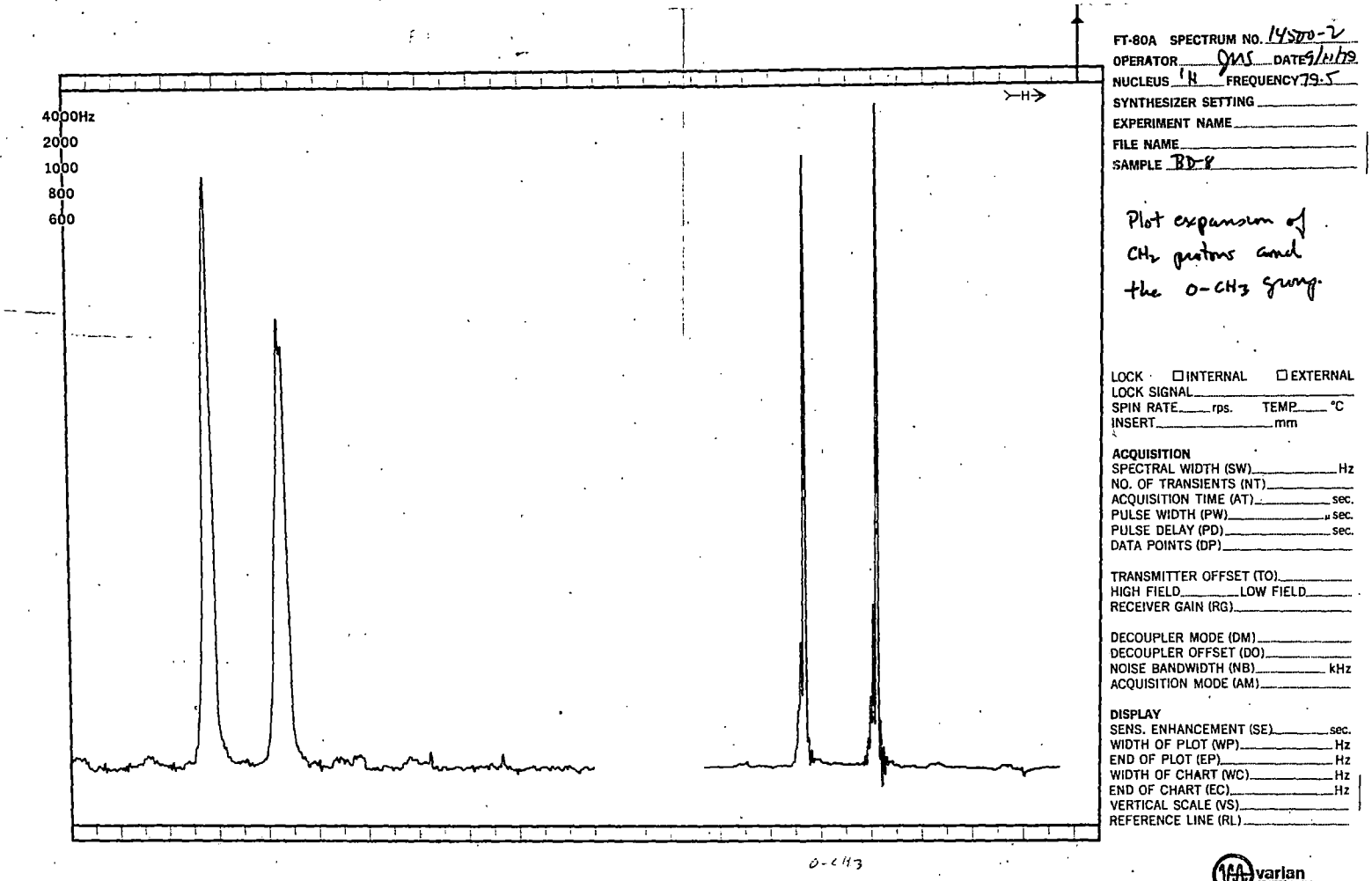


Fig. 48. Plot expansion of aromatic protons in BD-8



FT-80A SPECTRUM NO. 14500-2
OPERATOR GMS DATE 5/11/79
NUCLEUS 1H FREQUENCY 79.5
SYNTHESIZER SETTING
EXPERIMENT NAME
FILE NAME
SAMPLE BD-8

Plot expansion of CH_2 protons and the O-CH_3 group.

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

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

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

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

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

varian PRINTED IN U.S.A.

Fig. 47. Plot expansion of CH_2 protons and the O-CH_3 group in BD-8

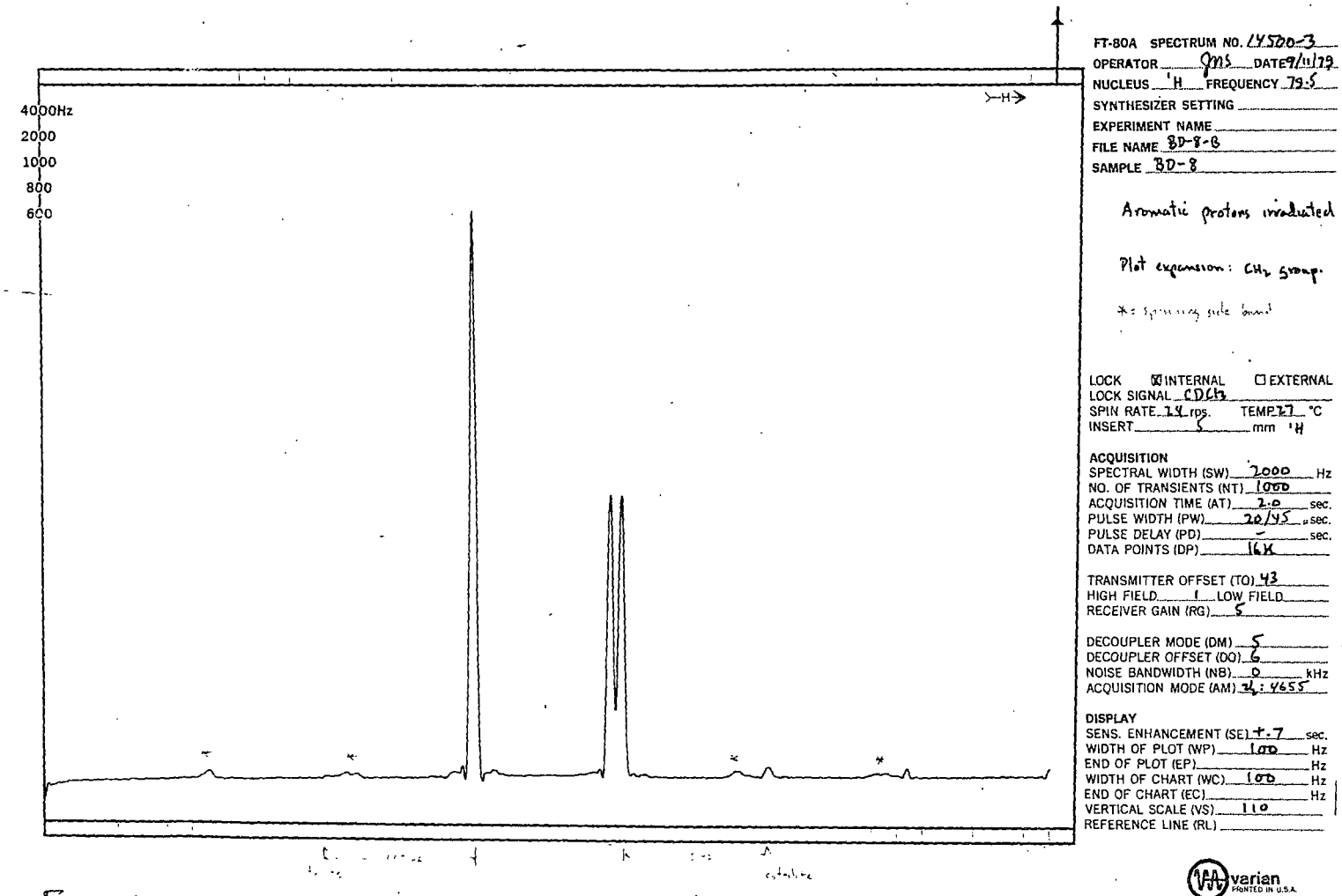


Fig. 48. Plot expansion: CH_2 group in BD-8 (aromatic protons irradiated)

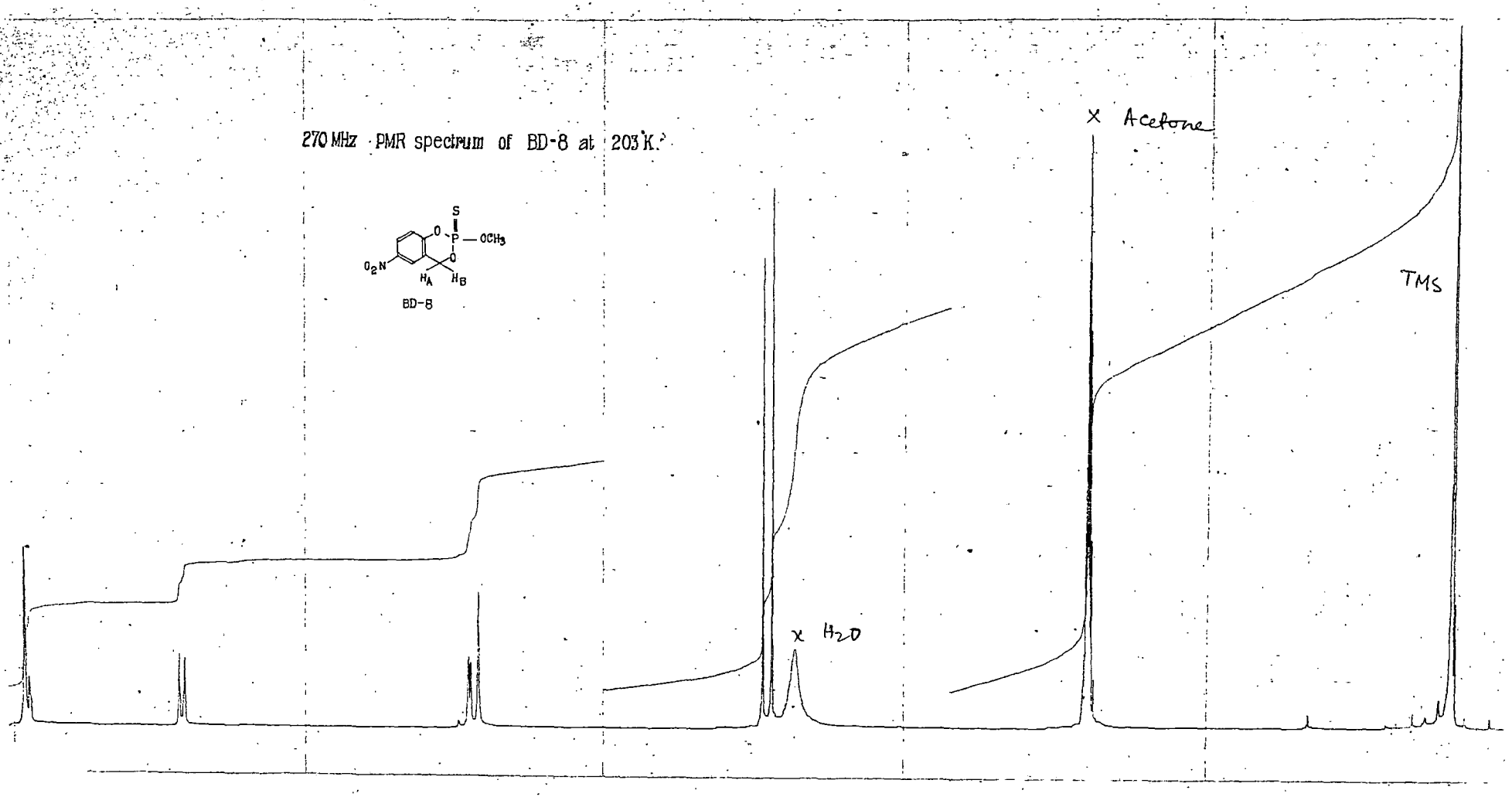
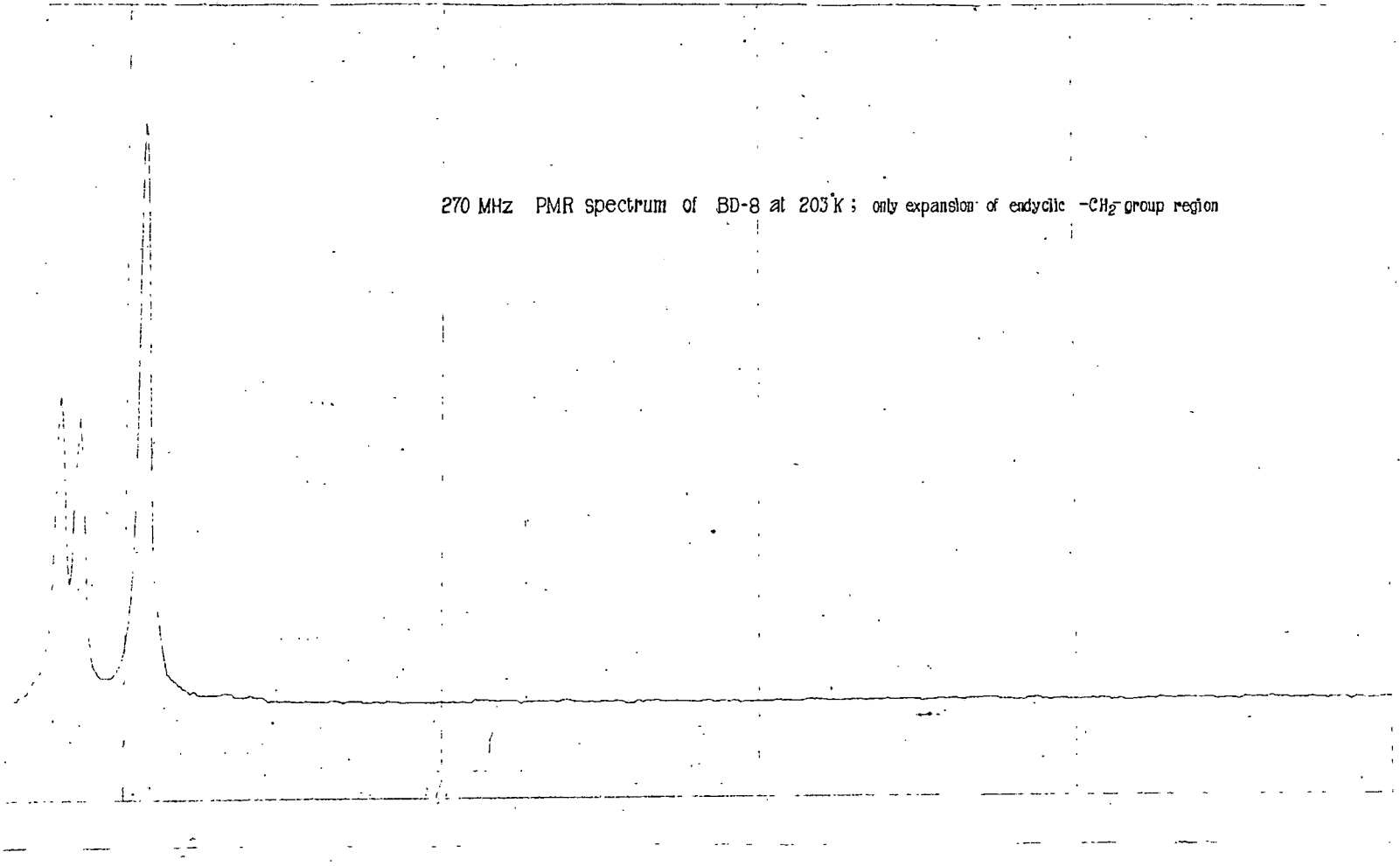

270 MHz PMR spectrum of BD-8 at 203°K.
S
O
P
OCH3
O
O2N
HA
HB
BD-8
x Acetone
TMS
x H2O

Fig- 49



270 MHz PMR spectrum of BD-8 at 203°K ; only expansion of endocic $-CH_2-$ group region

Fig - 50

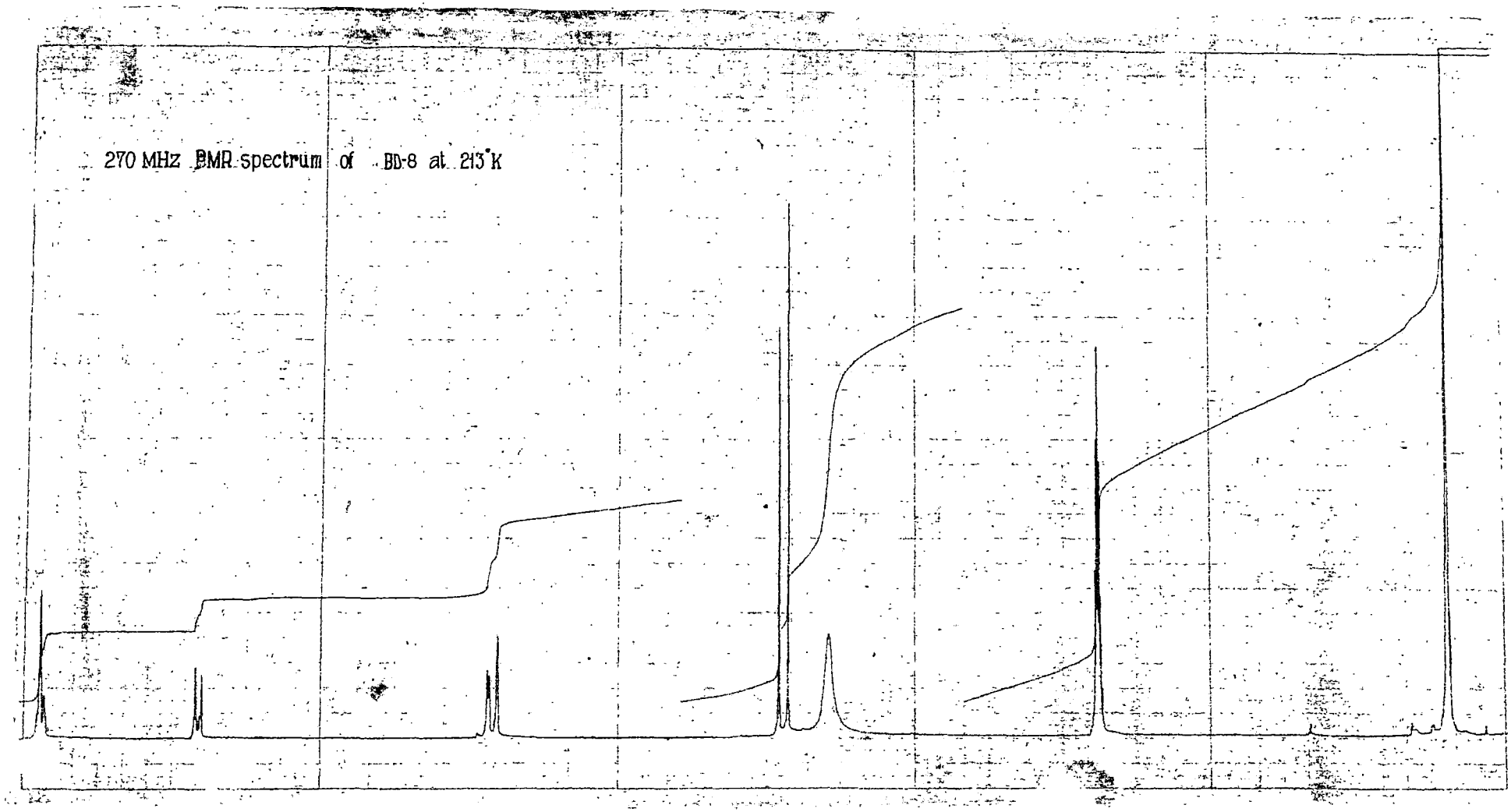


Fig-51

270 MHz PMR spectrum of BD-8 at 213°K ; only expansion of endocyclic $-CH_2-$ group region

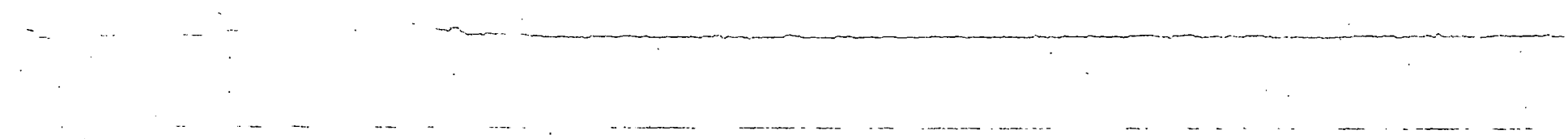


Fig- 52

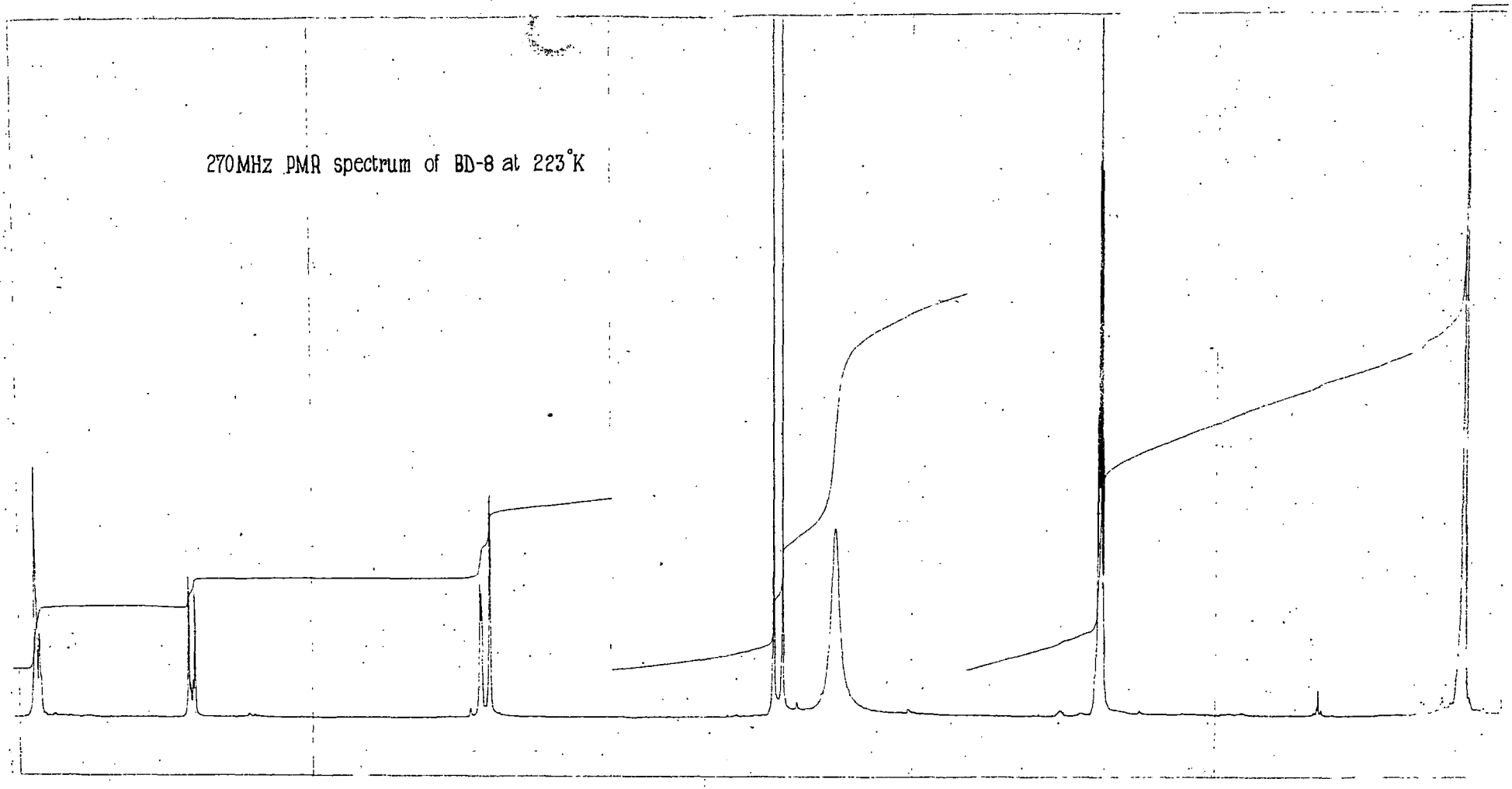


Fig - 53

270 MHz PMR spectrum of BD-8 at 223°K ; only expansion of endocyclic $-CH_2$ group region.

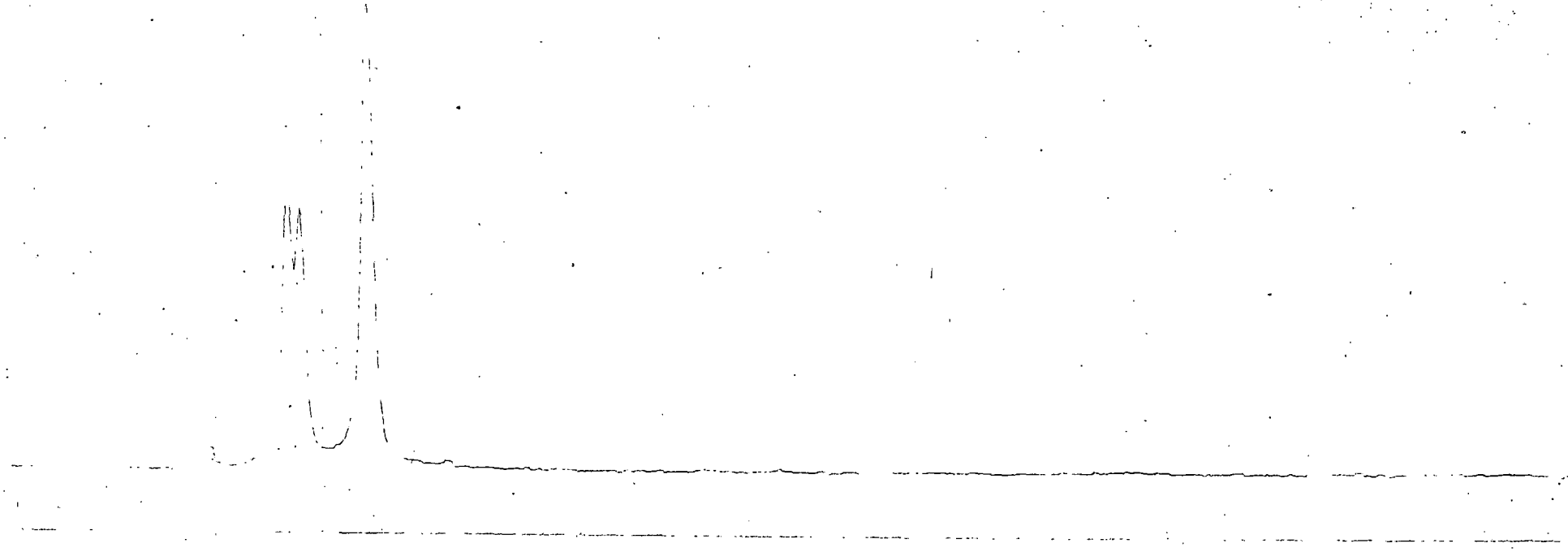


Fig - 54

70 MHz ... spectrum of BD-8 at 233°K.

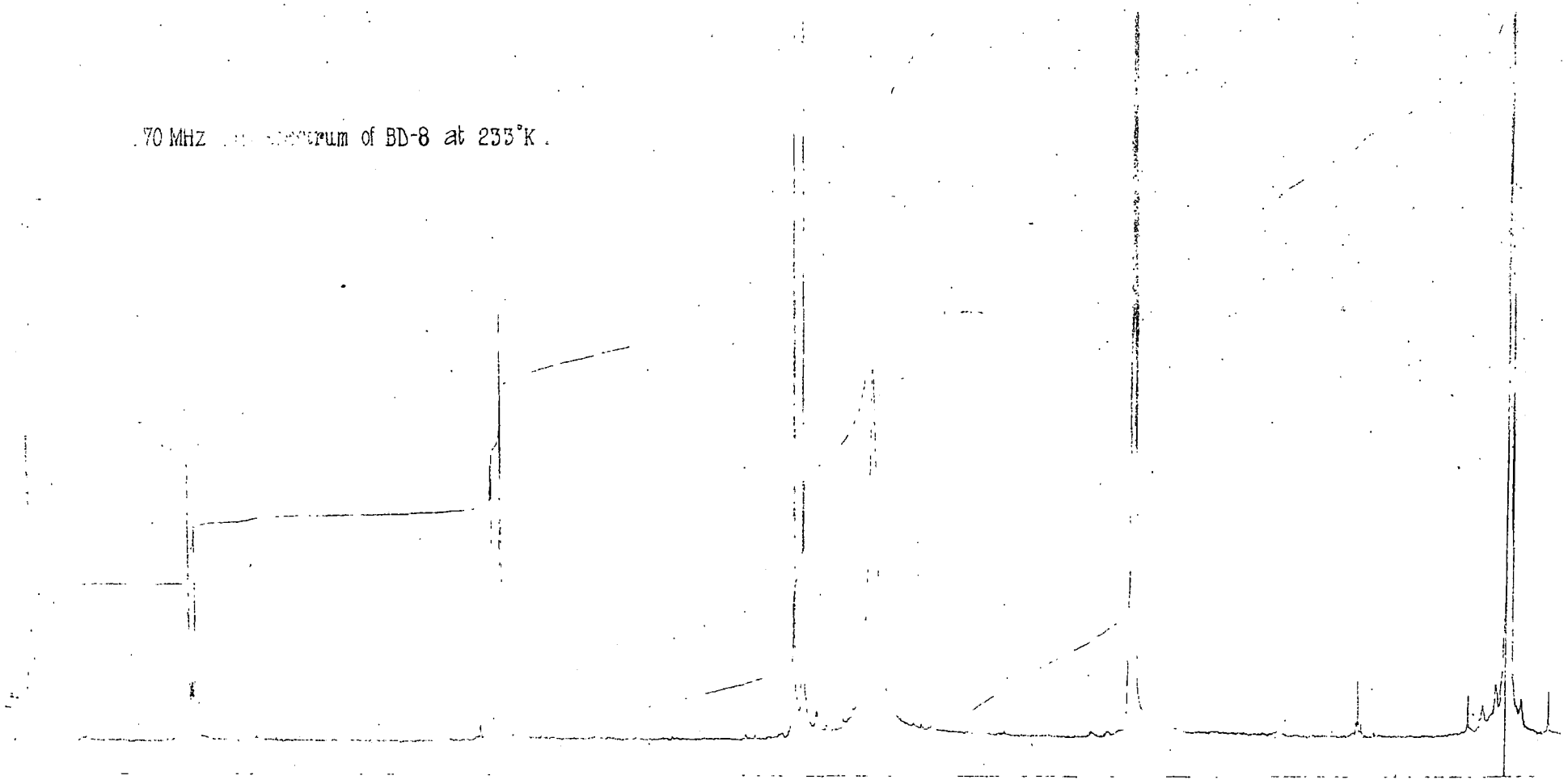


Fig - 55

270 MHz PMR spectrum of BB-8 at 233°K; only expansion of endocyclic $-CH_2-$ group region.

Fig - 56

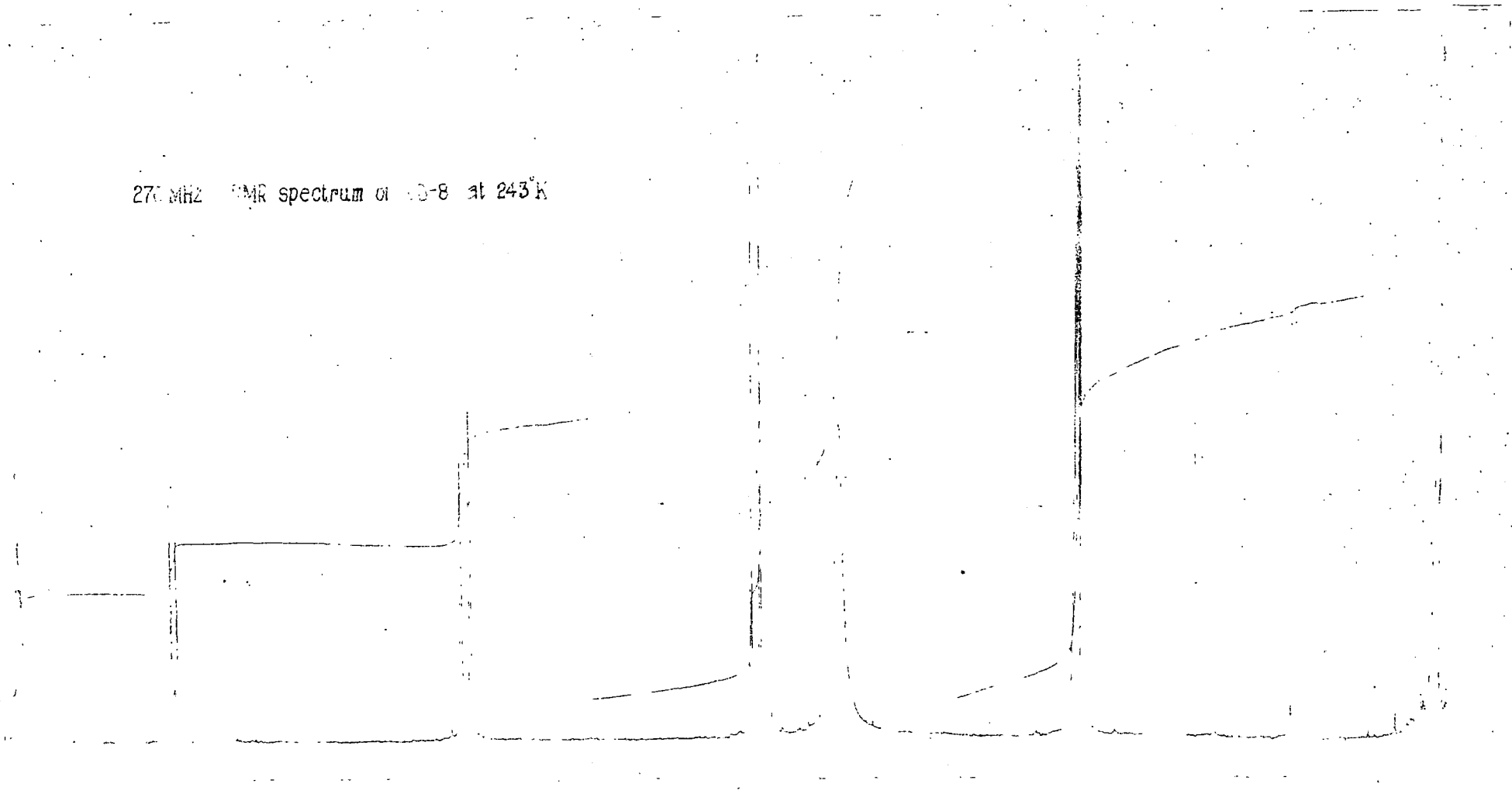


Fig-57

270 MHz PMR spectrum of BD-8 at 245°K ; only expansion of endocyclic $-CH_2-$ group region

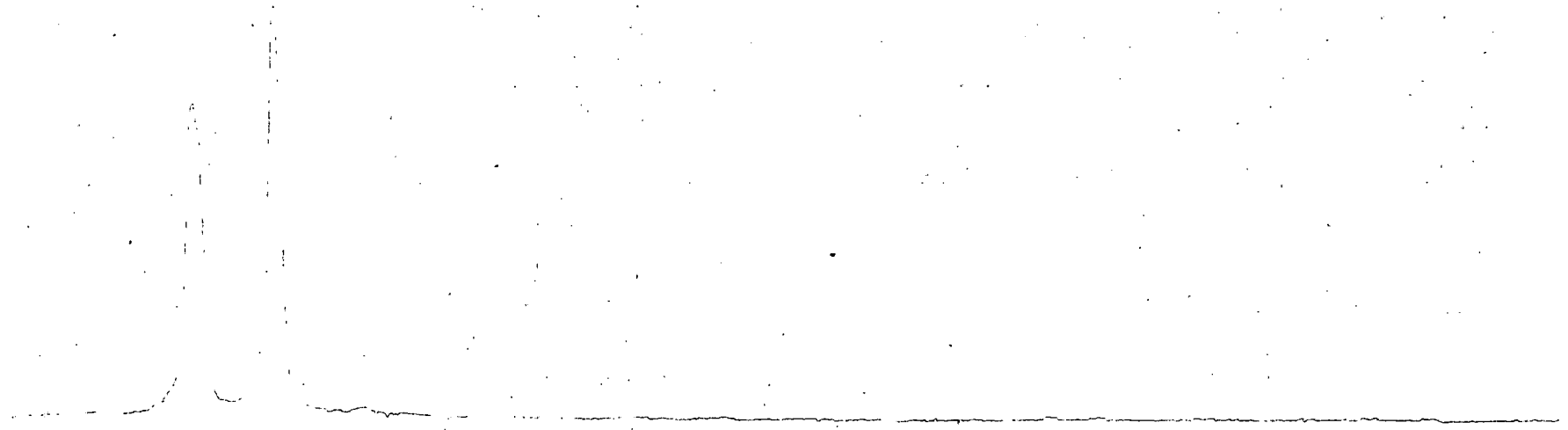


Fig- 58

270 MHz PMR spectrum of BD-8 at 253°K.

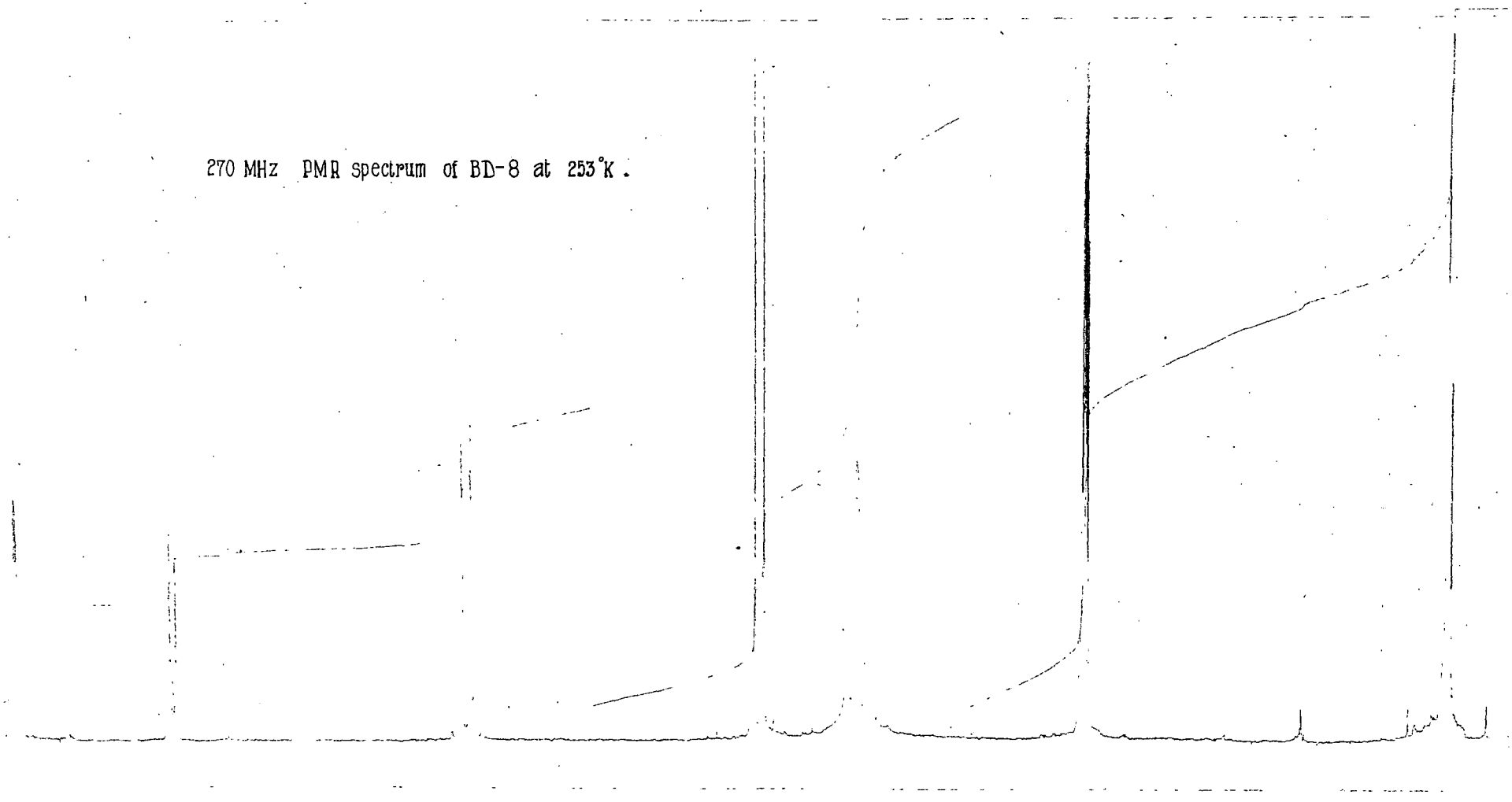


Fig - 59

270 MHz PMR Spectrum of BD-8 at 255°K ; only expansion of endocyclic $-CH_2-$ protons region

Fig- 60

270 MHz PMR Spectrum of BD-8 at 263°K

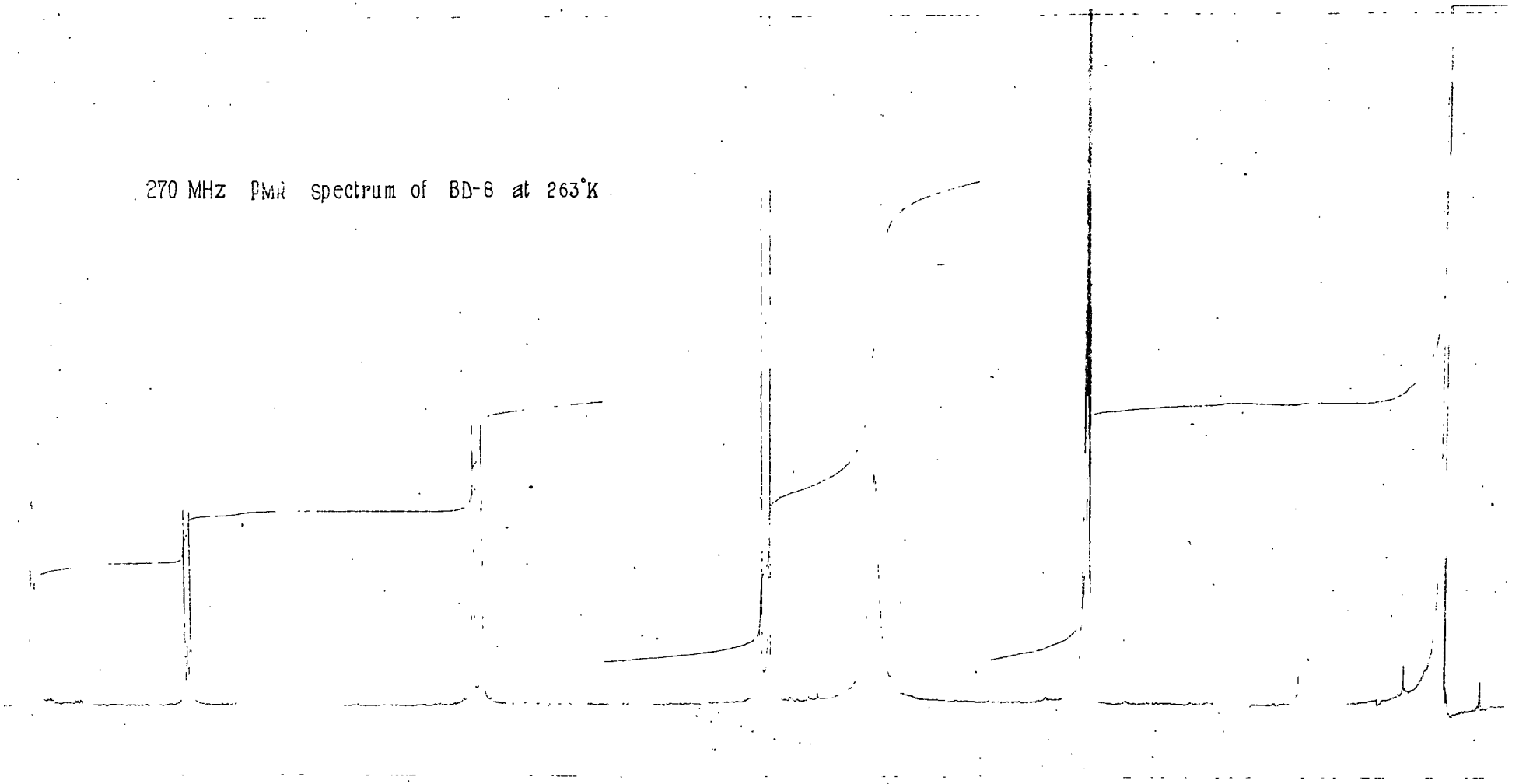


Fig- 61

270 MHz PMR spectrum of BD-8 at 263°K; only expansion of endocyclic $-CH_2-$ group region

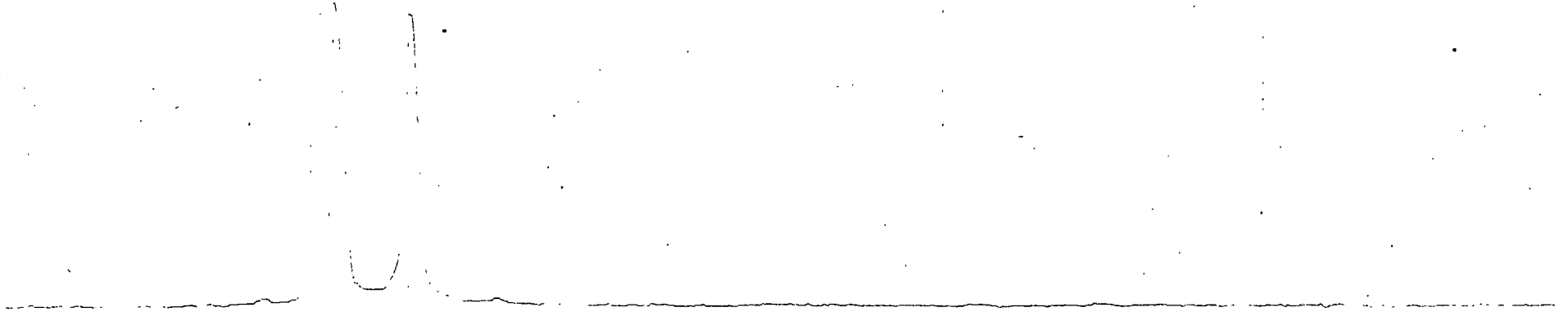
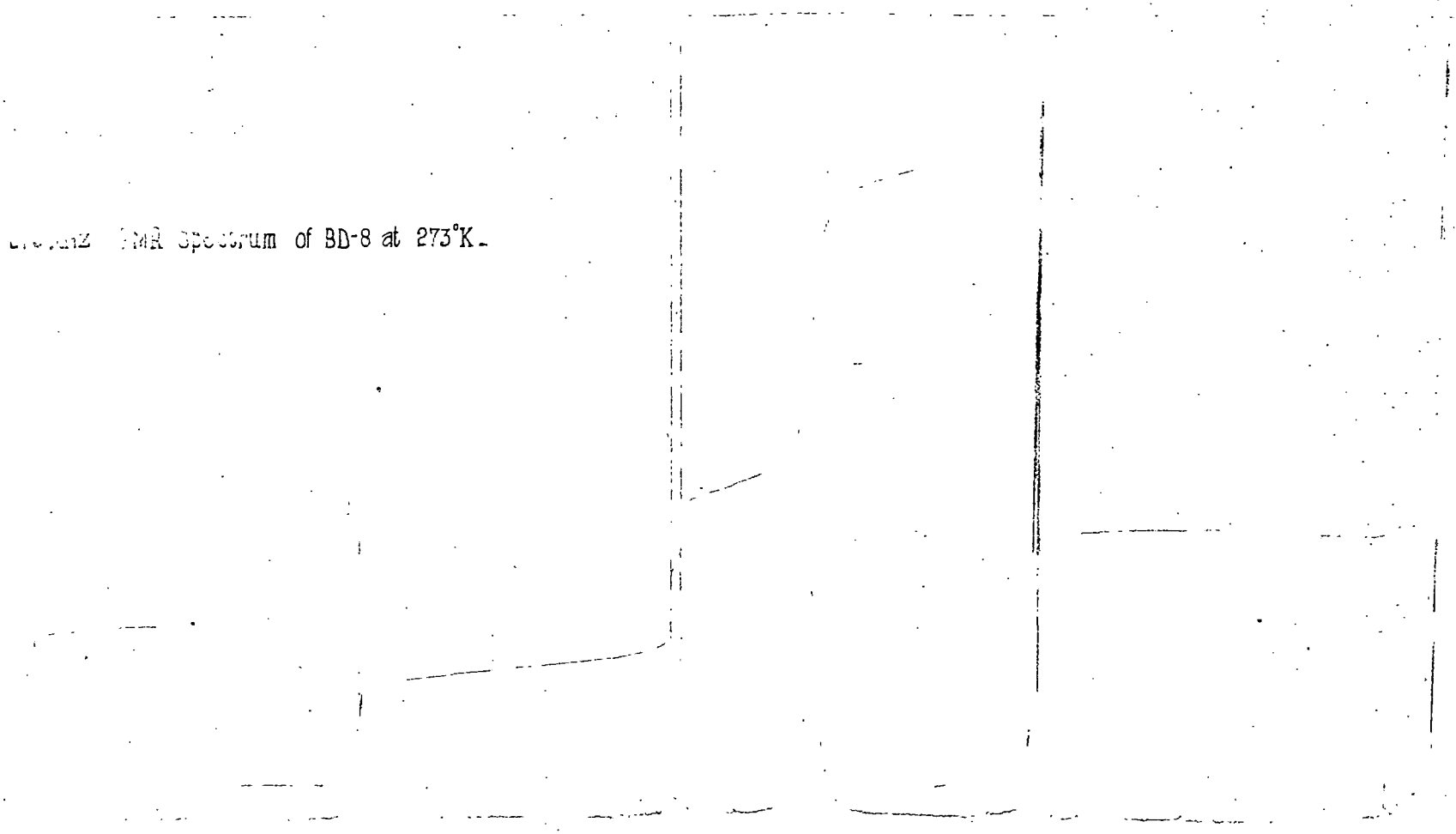


Fig- 62



100 MHz NMR Spectrum of BD-8 at 273°K.

Fig-63

270 MHz PMR spectrum of BD-3 at 273°K; only expansion of endocyclic $-CH_2-$ group region.

Fig- 64

270 MHz PMR spectrum of BD-8 at 283°K

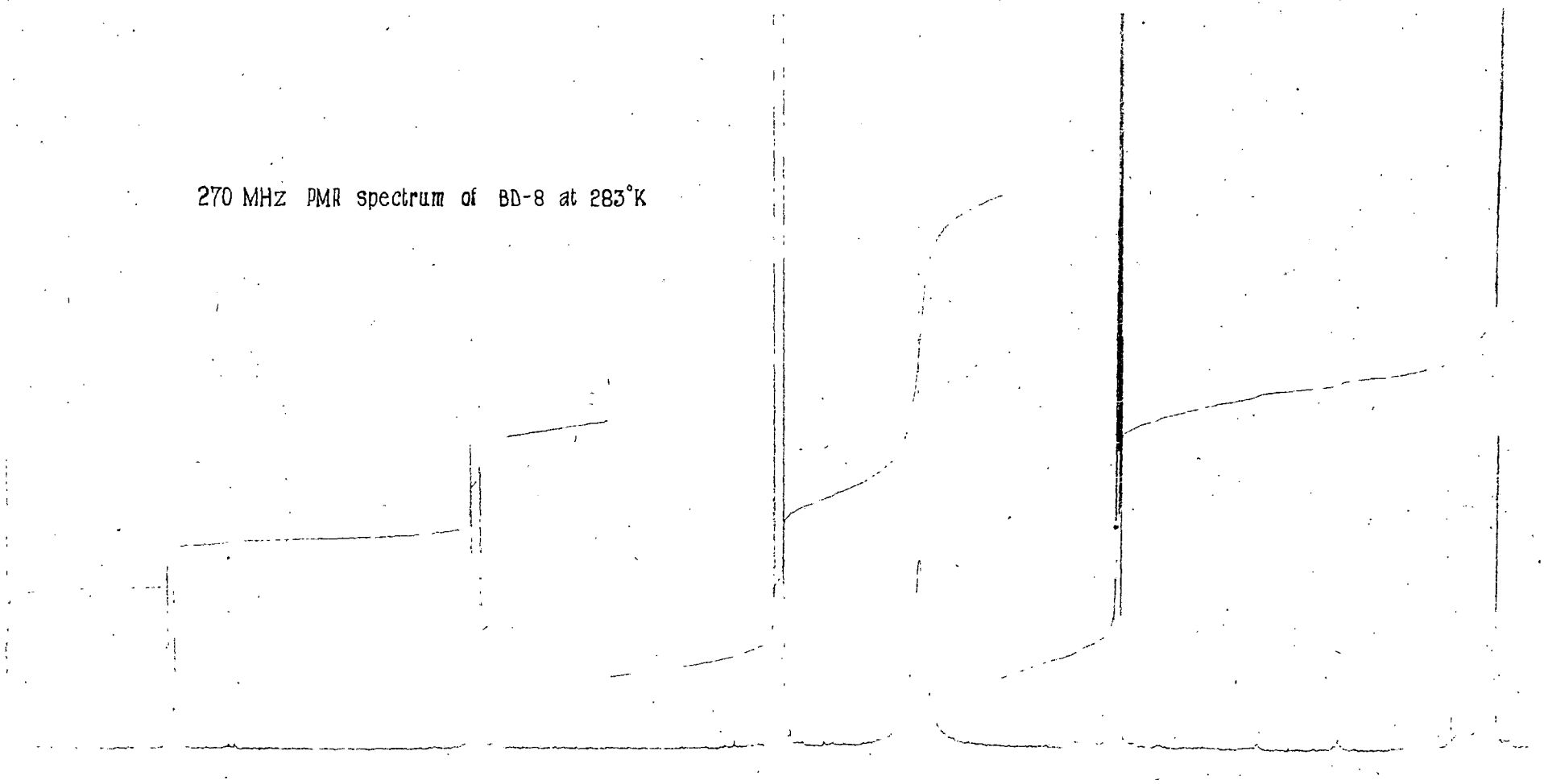


Fig- 65

270 MHz PMR spectrum of BD-8 at 283°K; only expansion of endocyclic $-CH_2$ group region.

Fig- 66

270 MHz PMR spectrum of BD-8 at 293°K

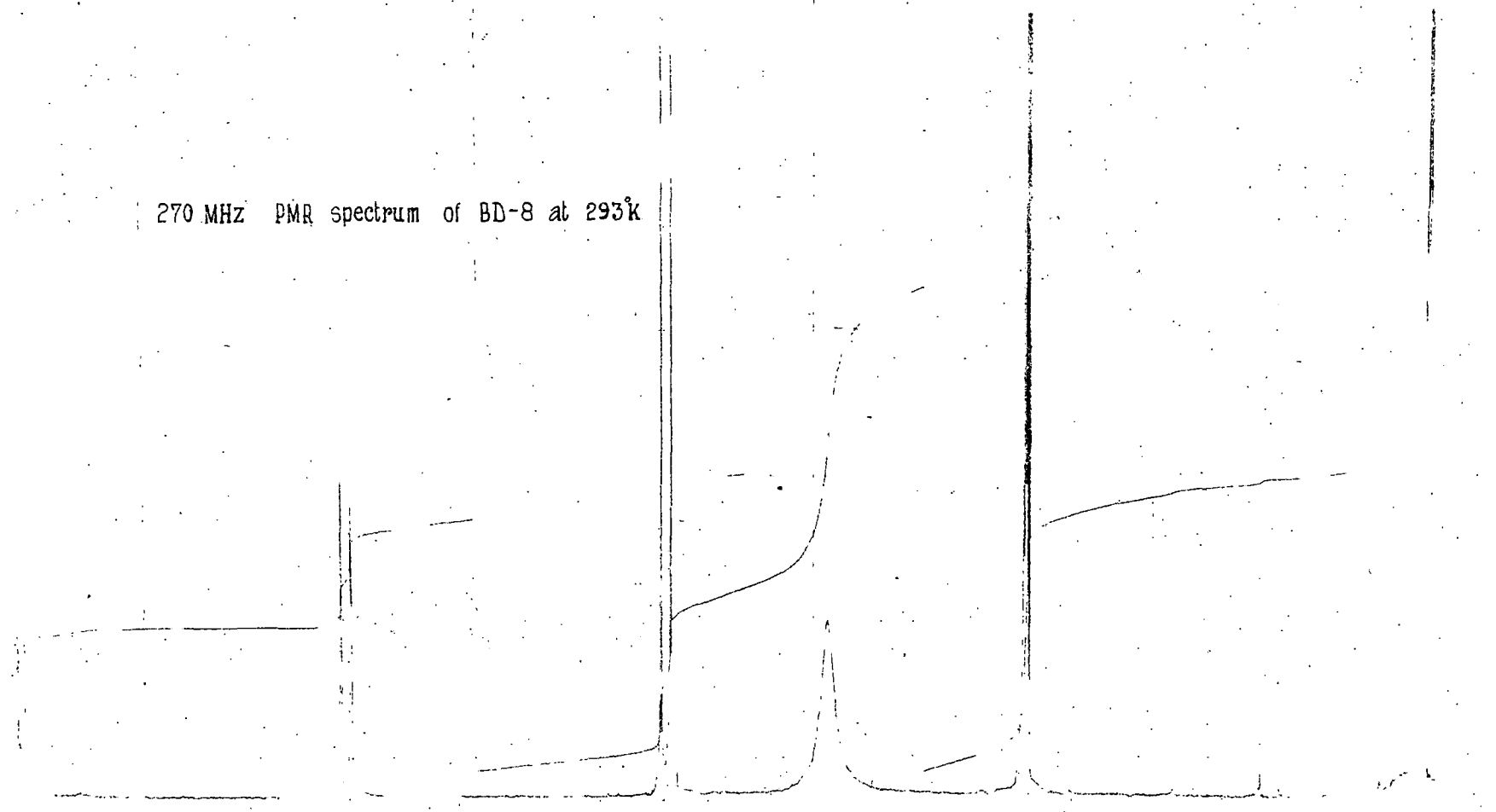


Fig- 67

270 MHz PMR spectrum of BD-8 at 293°K; only expansion of endocyclic $-CH_2-$ group region.

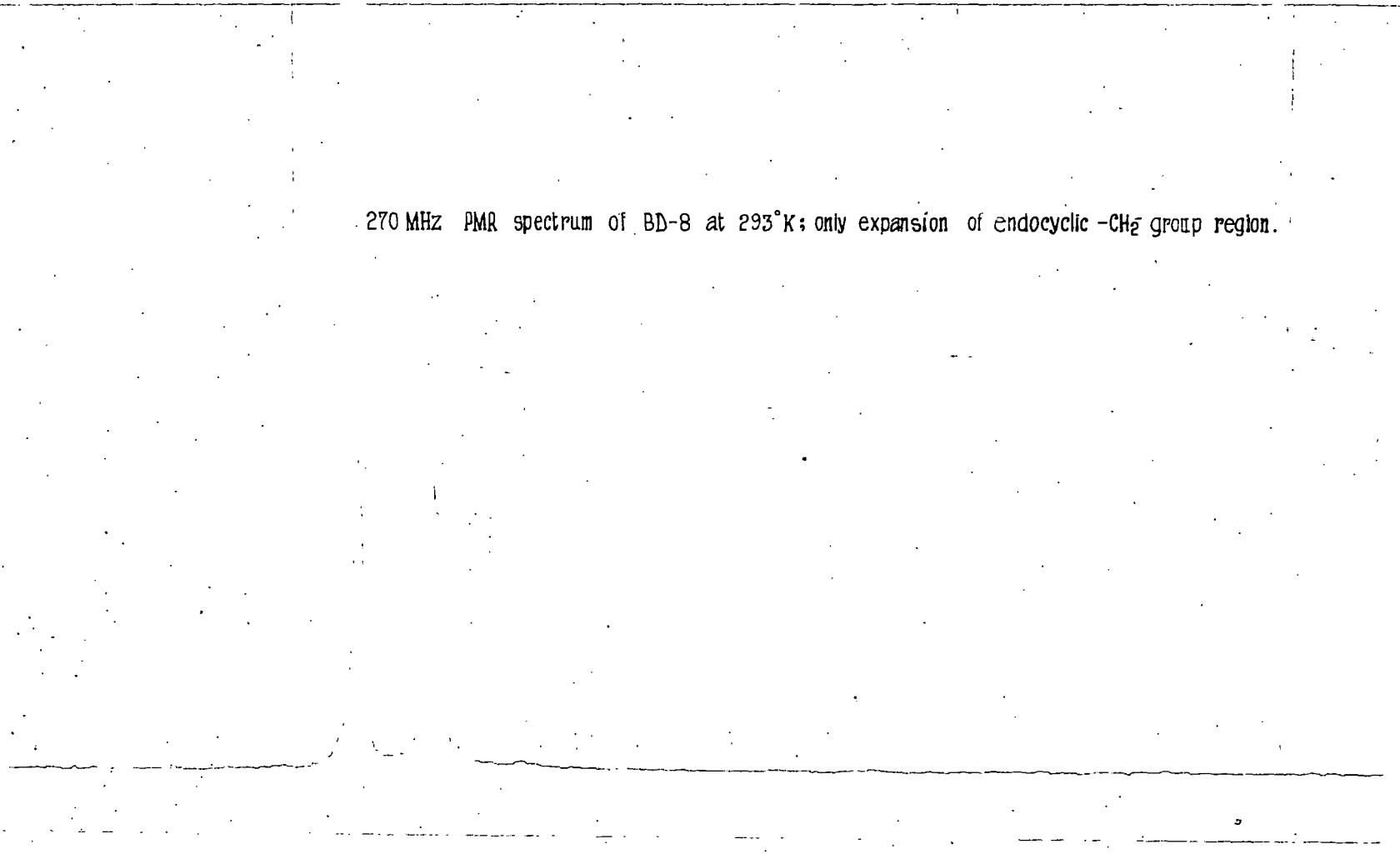


Fig - 68

270 MHz PMR spectrum of BD-8 at 303°K

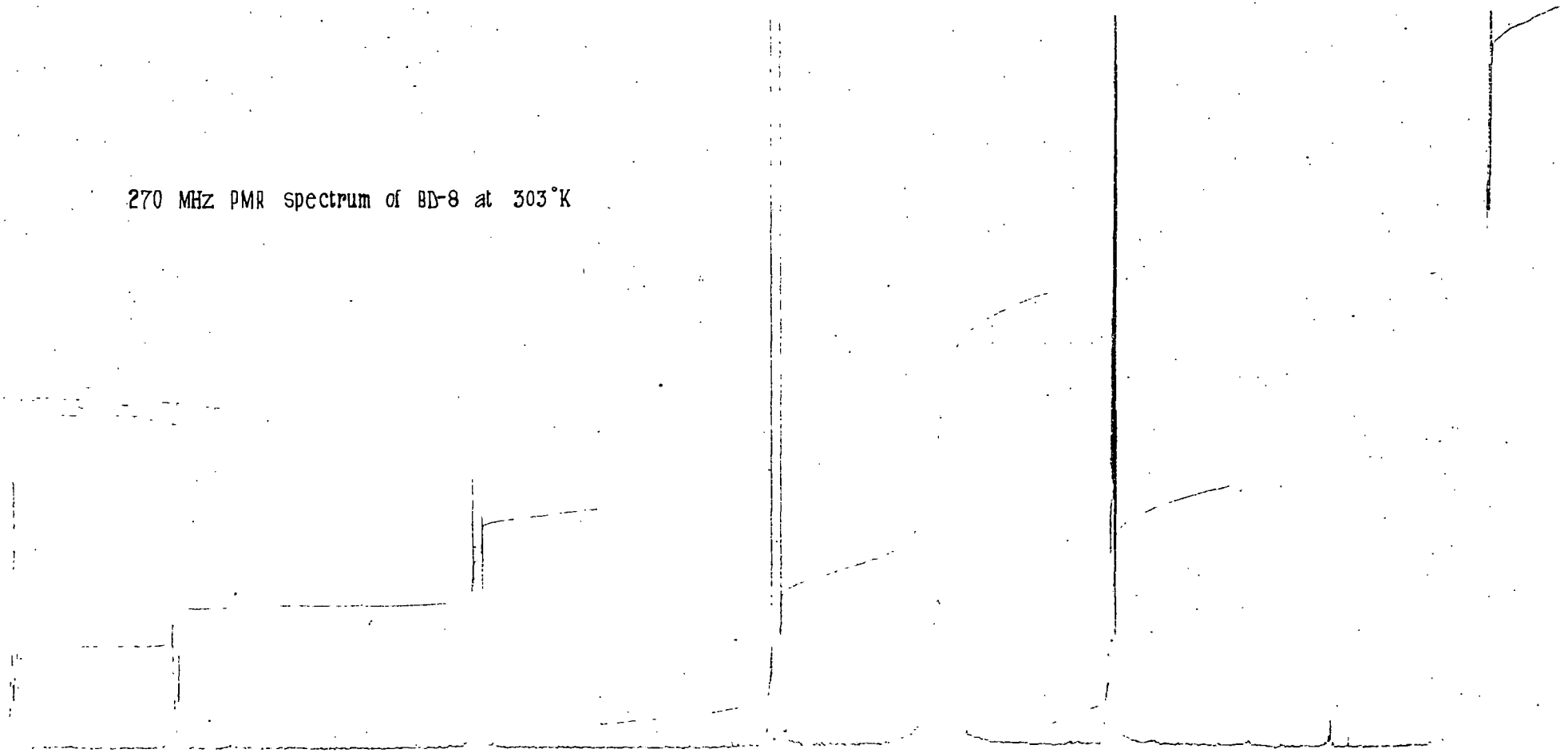


Fig - 69

270 MHz PMR spectrum of BD-8 at 303°K ; Only expansion of endocyclic $-CH_2-$ group region

Fig- 70

270 MHz PMR spectrum of [illegible]-B at 313°K

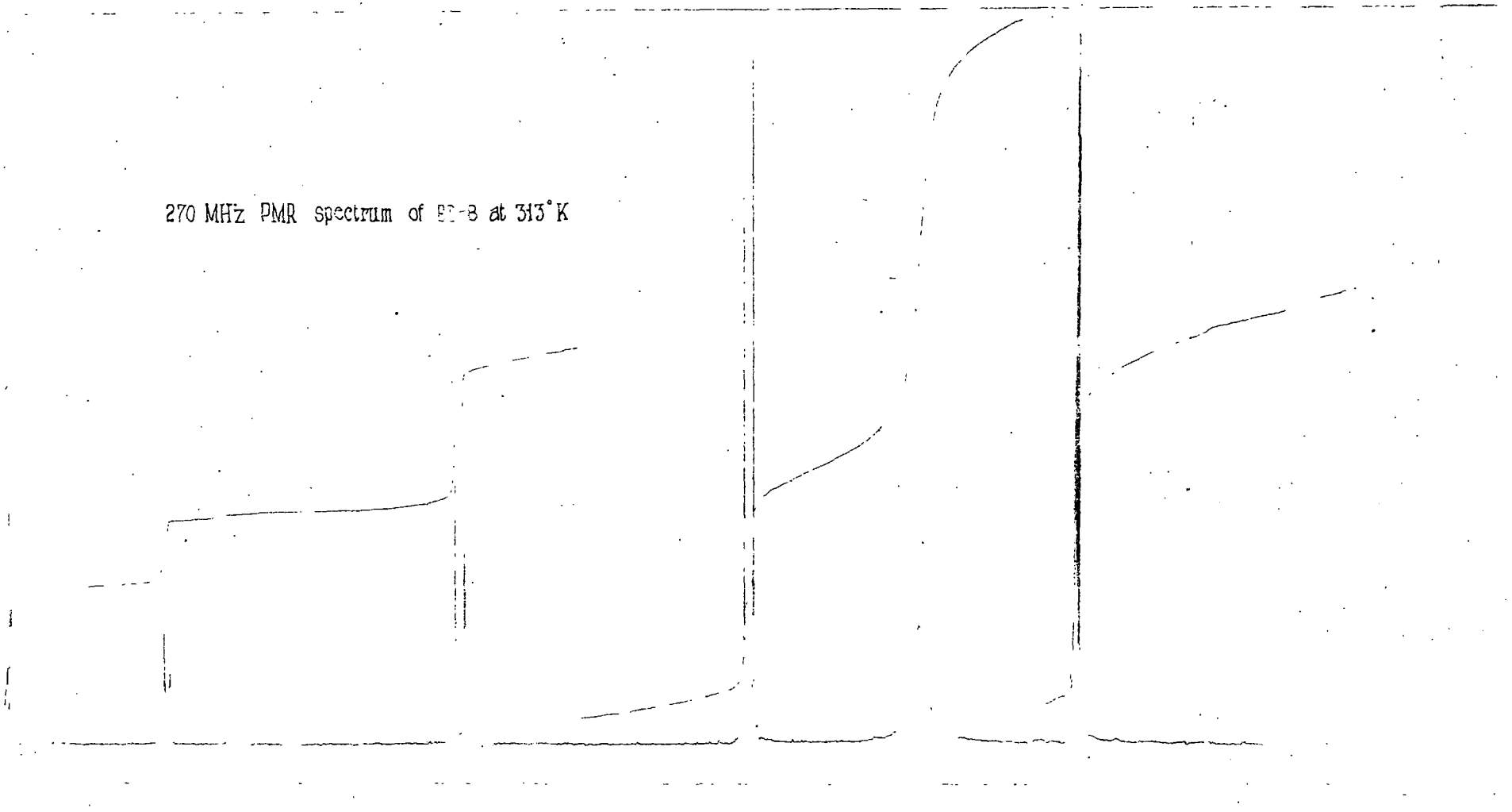


Fig- 71

270 MHz PMR spectrum of sD-8 at 313°K; only expansion of endocyclic $-CH_2-$ group region

Fig- 72

270 MHz PMR spectrum of BD-8 at 323°K.

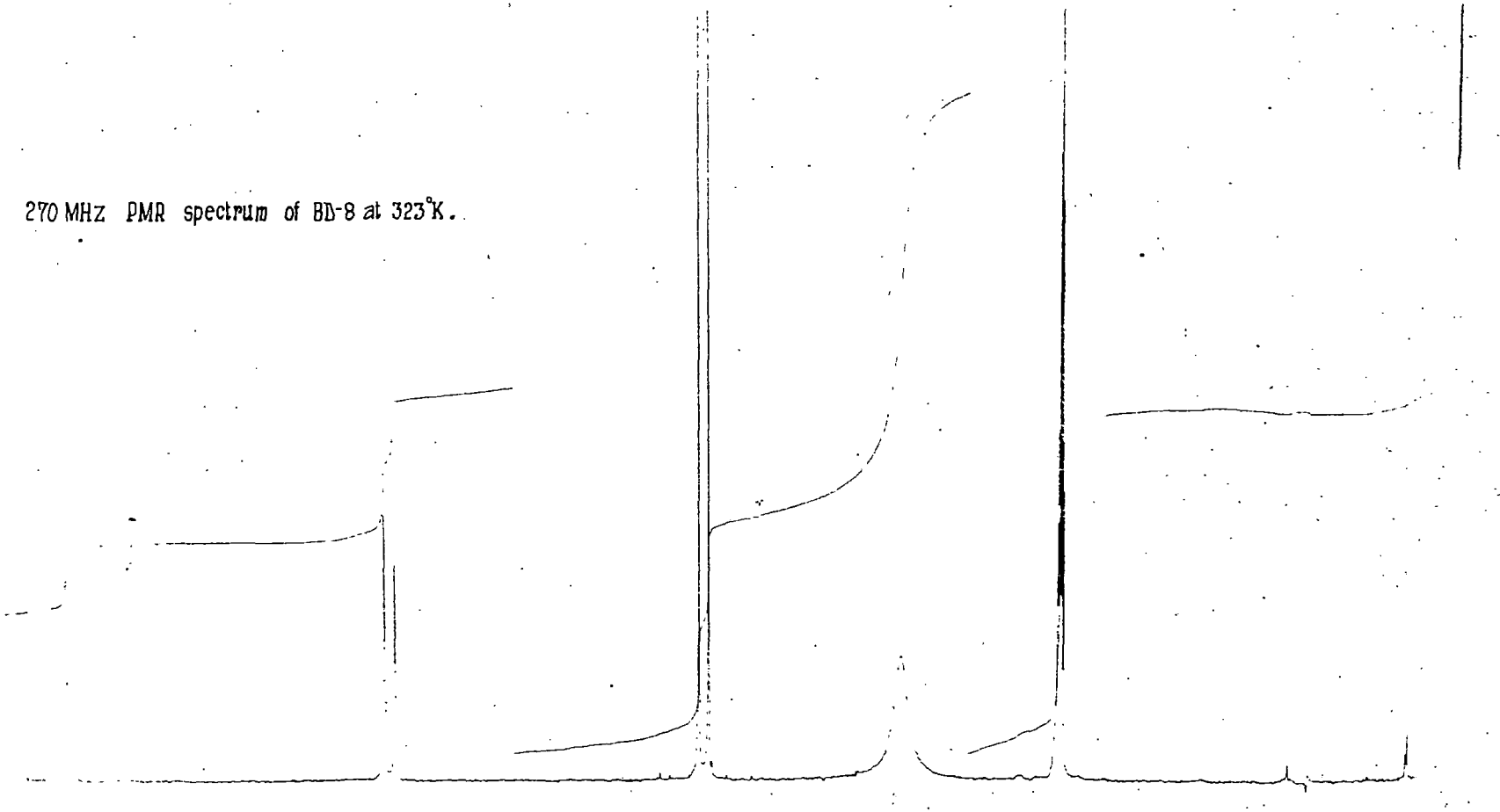


Fig- 73

270 MHz PMR spectrum of BD-8 at $323\,^{\circ}K$; only expansion of endocyclic $-CH_2-$ group region.

Fig- 74

interconversions of the conformers and their relative populations are also varied. It has also been observed that the signal at lower field of the doublet at 5.8 ppm splits further. This becomes much significant at -40°C (233°K); further splitting is observed at -70°C (203°K). This suggests that the methylene protons (H_{4A} & H_{4B}) are not equivalent to each other, and the dioxaphosphorin ring is conformationally mobile in solution.

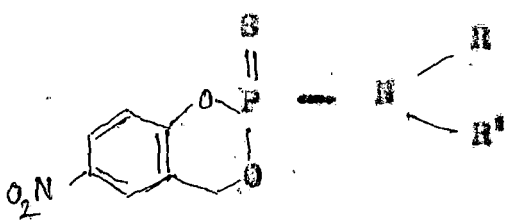
The ^{1}H NMR spectra of BD-10, and the ^{13}C, ^{31}P and ^{1}H NMR spectra of BD-15 and BD-16 seem to be in general accord with the structure II although they do not preclude another conformation (i.e., its inverted form, where oxygen and phosphorus lie below the plane of the aromatic ring). Further 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 relative to the $-CH_2-$ group in the dioxaphosphorin ring.

7. INSECTICIDAL ACTIVITY:

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

Table - 1

Insecticidal activity against Cockroaches, P. americana



Amido group	Code No.	Conc. showing 100% mortality (LC_{100}) (µg/gm)
Cyclohexylamido	BD-10	50 - 60
Morpholino	BD-11	30 - 40
Diethylamido	BD-12	30 - 40
Dimethylamido	BD-13	15 - 20
Iso-propylamido	BD-14	60 - 75
Pyrrolidino	BD-15	>75
Piperidino	BD-16	>75
Nonylamido	BD-17	>75
S P — OCH_3 O	BD-8	3 - 9
Salithion	-	8 - 13

The data presented in Table-I reveal that all alkylamidophosphorothionates have less oral insecticidal activity than the methoxy compound (BD-8) and salithion; BD-8 has greater insecticidal activity than salithion. Dimethylamido compound has only 1.5 to 2 times less insecticidal activity compared to salithion; morpholino and diethylamido compounds show 3 to 4 times less insecticidal activity compared to salithion. The other compounds are almost noninsecticidal. In whole series of nitro saligenin cyclic alkyl/phenyl/amido phosphorothionates prepared in our laboratory, only the methoxy compound (BD-8) has greater insecticidal activity than salithion. The dimethylamido compound has 2 times greater insecticidal activity compared to diethylamido compound. Eto et al [14] observed the same in case of 2-dimethylamido —, and 2-diethylamido-4H-1,3,2-benzodioxaphosphorin-2-sulphides. Among the alkylamidophosphorothionates (BD-10 - BD-17) the dimethylamido compound has highest oral insecticidal activity to roaches.

8. ANTIFUNGAL ACTIVITY:

Table-2 shows the antifungal activity data of these compounds (BD-10 - BD-16) against Helminthosporium Sp. ; the data for Hinosan (O - ethyl S,S - diphenyl-phosphorodithionate) have also been presented.

Table - 2

Antifungal activity of the alkylamidophosphorothionates against H. Sp.

Code No	Percent inhibition over control after 48 hours Concentration in µg/ml					
	500	250	200	150	100	50
BD - 10	100.0	70.3	63.5	59.5	46.0	33.0
BD - 11	83.3	76.7	74.2	70.0	65.0	54.9
BD - 12	100.0	74.0	64.7	53.5	42.2	22.0
BD - 13	90.9	73.0	69.7	59.5	51.5	34.8
BD - 14	100.0	100.0	100.0	90.0	74.0	64.8
BD - 15	84.0	72.0	68.0	62.0	54.0	42.0
BD - 16	100.0	79.4	73.5	65.0	54.4	41.2
Kinosan	100.0	96.7	90.3	82.2	65.3	51.0

The results reveal that all these compounds show inhibitory effect on the growth of Helminthosporium. sp. Complete inhibition of the growth is observed in case of BD-10, BD-12, BD-14 and BD-16 (at 500 μg/ml); however, other compounds are also effective at higher concentrations. The isopropylamidophosphorothionate (BD-14) is the most effective compound; its inhibitory effect is greater than that of Hinosan. At the concentration 60 μg/ml Hinosan shows 51.0 percent growth inhibition but BD-14 shows 64.8 percent inhibition. Only the compound BD-14 shows complete inhibition at the concentration 300 μg/ml, while other compounds show inhibition within the range 60 to 75 percent at the same concentration. Although BD-12 shows 100 percent inhibition at 500 μg/ml, but at 50 μg/ml it shows only 22.0 percent inhibition.

9. PHYTOTOXIC PROPERTIES:

The phytotoxicity data against Triticum Spp. (U.P. 302 variety) for the alkylamidophosphorothionates (BD-10 to BD-16) are listed in Table -3.

The effects of nitro-saligenin cyclic alkylamidophosphorothionates on germination of wheat seed (Triticum Spp.)

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

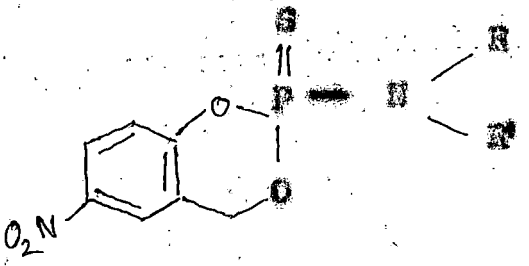
At 250 ppm and 100 ppm none of the compounds are phytotoxic to Triticum Spp. at 500 ppm BD-10 and BD-11 show slight phytotoxicity while other compounds are non-phytotoxic. In the case of BD-10 and BD-11 we observe 90 percent germination at 500 ppm.

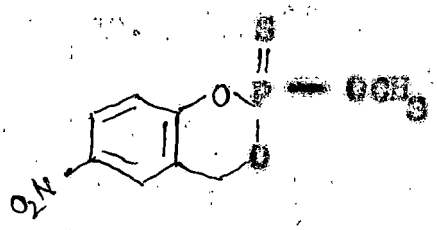
10. ACUTE ORAL TOXICITY ON RATS:

The acute oral toxicity data (LD_{50}) of the said alkylamidophosphorothionates on male rats are presented in table-4, and the results have been compared with salition and with the methoxy compound (BD-8).

Table - 4

Acute Oral toxicity on rats of the allylamidophosphorothionates



Amido group	Code No	LD_{50} (mg/kg) male rat
Cyclohexylamido	BD - 10	> 250
Morpholino	BD - 11	> 250
Diethylamido	BD - 12	150 - 250
Dimethylamido	BD - 13	125 - 250
Isopropylamido	BD - 14	> 250
	BD - 8	130 - 135
Sulithion	-	82

All compounds (BD-10 to BD-14) are less toxic than salithion. BD-12, BD-13 and BD-8 have greater toxicity to rats compared to other ketto saligenin cyclic phosphoramidothionates. Before death the rats were found to suffer from acute respiratory trouble. In some cases, a fluid with blood-stain oozed out of nostrils and eyes of the animals. In all cases, the decrease of spontaneous motor activity occurred after 2-4 hours. Salivation and irregular respiration were observed. In case of morpholino compound the colour of the rats became yellow after 20-30 hours; the yellow colour disappeared after 3-5 days. No other compounds showed this symptom.

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

11. ANTICHOLINESTERASE ACTIVITY:

The acetylcholinesterase inhibition data for housefly-head homogenate (HFAChE) and goat whole blood (blood - ChE) are listed in Table-5A - 5E (pp 187-191) and Table - 6A - 6E (pp 192-196) respectively; the molar I_{50} values calculated by least square programme are given below (Table-5). The data for only five compounds have so far been taken.

Table - 5

Anticholinesterase activity on housefly head homogenate and goat whole blood.

Code No.	Amido Group	I_{50}(M) x 10^5 (HFAChE) housefly	I_{50}(M) x 10^4 (ChE) goat whole blood
1	2	3	4
BD - 10	Cyclohexyl amido	2.86	1.81
BD - 11	Morpholino	5.43	1.04
BD - 12	Diethylamido	8.26	1.43
BD - 13	Dimethylamido	1.80	4.66
BD - 14	Isopropylamido	1.86	4.78

It has been observed that for any phosphoramidothionate (BD-10 to BD-14), the HFAChE is more inhibited than the ChE-blood. For the HFAChE, the I_{50} value increases in the order:

BD-13 < BD-14 < BD-10 < BD-11 < BD-12

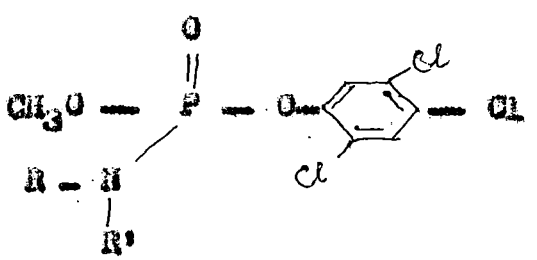
i.e, the antiacetylcholinesterase activity of the dimethylamido-phosphorothionate is highest, and that of the diethylamido analog is least. For the ChE-blood, the I_{50} value increases in the order:

BD-11 < BD-12 < BD-10 < BD-13 < BD-14

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

From the above results it may be concluded that the dimethylamido compound has good insect-acetylcholinesterase inhibitory activity, but the isopropylamido compound has least toxic effect on mammalian blood-cholinesterase. Previously it has been observed that the dimethylamido compound has also insecticidal activity (among the eight alkylamidophosphorothionates it has highest insecticidal activity), but the isopropylamido compound has highest antifungal activity.

Hansch and Deutsch[27] analysed the data obtained by Fukuto et al[28] from a series of methyl 2,4,5 - trichlorophenyl-N-alkyl phosphoramidates in order to clarify the effect of the



N-alkyl group on the inhibitory activity for the $HF^{A}ChE$. They observed that the logarithm of the bimolecular inhibition constant is correlated excellently with Taft's steric constant E_s and polar constant $\overset{*}{\sigma}$ of the substituent; the bulky isopropyl and tert-butyl groups decrease inhibition rates by steric interference. On the other hand, the ring substituents of methyl phenyl N-methyl phosphoramidates directly affect the antiacetylcholinesterase

activity (HFAChE) by virtue of the electronic and hydrophobic properties.[29] However, in a series of ethyl S-(substituted)-phenyl phosphoramidothiolates, no correlation was observed between the rates of cholinesterase inhibition and any of the free energy parameters for ring substituents[30]; moreover, the anticholinesterase activity (HFAChE) of phosphoramidothiolates is not always correlated with their insecticidal activity.

In case of nitro-saligenin cyclic phosphoramidothionates antiacetylcholinesterase activity is not correlated with their insecticidal activity. Among the five compounds (BD-10 to BD-14), only dimethylamido compound (BD-13) shows highest insecticidal activity, and also highest antiacetylcholinesterase activity. Although antiacetylcholinesterase activity of both dimethylamido and isopropylamido compounds are comparable (I_{50} is 1.80×10^{-5}M and 1.86×10^{-5}M respectively, Table-5, Column-3), the insecticidal activity of the dimethylamido compound is highest (LC_{100}=15-20 g/g) and that of the isopropylamido compound is least (LC_{100}=60-75 g/g). When the data for other nitro-saligenin cyclic phosphoramidothionates will be available we will try to find out the correlation between antiacetylcholinesterase activity and E_s, σ^* as well as π values of alkylamido groups. Previous studies[31] in this laboratory concerning the biological activities of some 2-alkoxy-6-nitro-4H-1,3,2-benzodioxaphosphorin-2-sulphides, a good correlation, as shown in the folloiwng equations, has been obtained between the anti-AChE activity (housefly) and σ^*, E_s^c as well

as π values:

$$-\log I_{50} = 4.9057\ \overset{*}{\sigma} + 7.3703 \quad \ldots\ldots\ldots \quad (1)$$

$$n = 6,\ r = 0.8142,\ s = 0.3413$$

$$= 0.8766\ E_s^c + 7.1550 \quad \ldots\ldots\ldots \quad (2)$$

$$n = 6,\ r = 0.7784,\ s = 0.3413$$

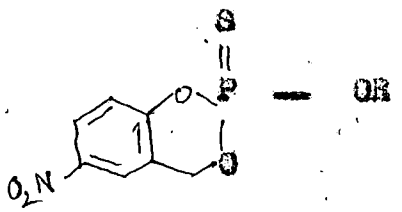
$$= -0.6737\ \pi + 7.7312 \quad \ldots\ldots\ldots\ldots \quad (3)$$

$$n = 6,\ r = 0.9351,\ s = 0.3413$$

$$= 1.7032\ \overset{*}{\sigma} + 0.0825\ E_s^c - 0.4781\ \pi + 7.6913 \ldots (4)$$

$$6 = 6,\ r = 0.9564,\ s = 0.3413$$

where, n is the number of data points used in the regression, r is the correlation co-efficient, and s is the standard deviation. Equation 4 gives the best fit to the experimental data. However, equation 3 also gives good fit as judged by correlation co-efficient value. These equations (3 & 4) show the great importance of the relative hydrophobic binding constant (π-value) of the exocyclic alkyl groups. By using equation 4 we have calculated the value of $-\log I_{50}$, and the results have been presented in the following table:



R	-log I_{50}(Calculated)	-log I_{50} (observed)
CH_3	7.4427	7.534
C_2H_5	7.0220	6.863
n-C_3H_7	6.7492	6.787
i-C_3H_7	6.5985	6.756
n-C_4H_9	6.4967	6.572
i-C_4H_9	6.5031	6.472

As mentioned earlier when the data for other cyclic amidophosphorothionates will be available we will try to find out the correlation between IIFAChE and E_s, as well as .

12. CHEMICAL HYDROLYSIS:

The alkaline hydrolysis for some alkylamidophosphorothionates have been carried out in 0.0095 M NaOH (in 50% ethanol) at 20°C. The hydrolysis data for each compound at different wave lengths (λ =400, 410, 420 and 430 nm) are listed in the tables 7A to 11 B (pp 197 - 214); the values of hydrolysis constant have been calculated by least square regression programme. The hydro-

lysis data have been summarised below (Table-7):

Table - 7

Hydrolysis of nitro-saligenin cyclic amidophosphorothionates; pH 11.85 (50% Ethanol), Temperature = 20°C

Code No	Amido Group	λ in mμ	K_{hyd} (min^{-1})	Average K_{hyd} (min^{-1})	$T_{1/2}$
1	2	3	4	5	6
BD-10	Cyclohexylamido	400	10.824×10^{-3}	10.364×10^{-3}	67 min
		410	10.364×10^{-3}		
		420	10.364×10^{-3}		
		430	9.903×10^{-3}		
BD-11	Morpholino	400	6.1413×10^{-5}	6.1413×10^{-5}	11286.6 min.
		410	6.1413×10^{-5}		
		420	6.1413×10^{-5}		
		430	6.1413×10^{-5}		
BD-13	Dimethylamido.	400	2.687×10^{-5}	2.878×10^{-5}	23896.5 min
		410	2.687×10^{-5}		
		420	3.07×10^{-5}		
		430	3.07×10^{-5}		
BD-17	Nonylamido	400	20.266×10^{-3}	19.4025×10^{-3}	35.72 min
		410	20.266×10^{-3}		
		420	19.345×10^{-3}		
		430	17.733×10^{-3}		
BD-15	Pyrrolidino	400	1.7835×10^{-5}	1.7632×10^{-5}	39500 min.
		410	1.7784×10^{-5}		
		420	1.7452×10^{-5}		
		430	1.7466×10^{-5}		
BD-16	Piperidino	400	1.0952×10^{-5}	1.162×10^{-5}	43312 min.
		410	1.2129×10^{-5}		
		420	1.1541×10^{-5}		
		430	1.1856×10^{-5}		

The average hydrolysis constants recorded in Table - 7 (Column-5) show that the nature of the amido group in the exocyclic side chain influences the stability of the compounds to alkaline hdyrolysis. The K_{hyd} increases in the order:

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

i.e. the piperidino compound (BD-16) is most stable, and the nonylamido compound (BD-17) is least stable to alkaline hydrolysis. The cyclic phosphoramidothionates containing the disubstituted amido groups (piperidino, extremely resistant to hydrolysis compared to the other compounds having the monosubstituted amido groups (nonylamido and cyclohexylamido); probably the steric-interferences of the cyclohexylamido and nonylamido groups are less compared to that of the pyrrolidino, piperidino, dimethylamido and morpholino groups.

The hydrolysis of the saligenin cyclic esters proceeds with the initial fission of the endo-cyclic ester bond, and the cleavage of the exocyclic ester bond does not take place by alkaline hydrolysis (13). In our case, we could not detect any free amine in alkaline medium, and hence there is no cleavage in the P - N bond. Following the work of Eto (Ref. 13, p - 74, Equation-34) it may be proposed that the following reaction is taking place in course of alkaline hydrolysis:

Contrary to alkali hydrolysis in 0.0005 M NaOH these phosphoramidothionates show a good deal of resistance to the hydrolysis at pH 7.7 in phosphate buffer. For example, no detectable hudrolysis occurs even after 36 hours in case of morpholino, dimethylamido, pyrrolidino and piperidino compounds. However, the nonylamido and cyclohexylamido compounds show slight hydrolysis at pH 7.7 in phosphate buffer; the rate of hydrolysis (at pH 7.7) is less than that at pH 11.85. Therefore, it may be concluded that the rate of alkali hydrolysis is increased as the pH value increases from 7.7 to 11.85.

Salioxon, salithion and nitro-salithion (BD-8) which have been found as high insecticidal compounds in the series of saligenin cyclic phosphorus esters are rather unstable. The cyclic phosphoramidothionates presented here are more stable.

13. GENERAL CONCLUSIONS AND REMARKS:

(i) The dimethylamido compound (BD-13) shows greater insecticidal activity than other compounds to roaches; however, it has 1.5 to 2 times less insecticidal activity compared to salithion. The other compounds are non-insecticidal.

(ii) All compounds show antifungal activity against Helminthosporium spp. However, the isopropylamido compound (BD-14) is the most effective compound; its inhibitory effect is greater than that of Kinosan.

(iii) Except the cyclohexylamido (BD-10) and the morpholino (BD-11) compounds, none of the compounds are phytotoxic to Triticum spp. upto the concentration 500 ppm (the highest concentration used).

(iv) All compounds (BD-10 to BD-14) are less toxic to rats than salithion.

(v) From anticholinesterase activity studies it has been observed that for any phosphoramidothionate (BD-10 to BD-14), the HFAChE (housefly) is more inhibited than the ChE - blood. For the HFAChE, the activity of the dimethylamidophosphorothionate is highest, and that of the diethylamido analog is least. For the ChE - blood, the anticholinesterase activity of the morpholino compound is most, and that of the isopropylamido compound is least. It may be concluded that the dimethylamido compound has good insect-acetylcholinesterase inhibitory activity (it has also good insecticidal activity), but the isopropylamido compound has least toxic effect on mammalian blood - cholinesterase (it has good fungicidal activity).

(vi) From the chemical hydrolysis studies it has been observed that the compounds containing the disubstituted amido groups are extremely resistant to hydrolysis compared to other compounds having the mono-substituted amido groups.

(vii) All compounds show common IR bands due to P-O-C (alkyl), P-O-C (aryl), P=S, NO_2 groups etc. Although P-H absorption shows considerable frequency shifts, the N-H stretching vibrations occur in the normal frequency region.

(viii) All compounds show parent molecular ions. Fragmentation by loss of SH radical is important; all compounds show an ion due to (parent molecule - .SH)$^+$ and it is the base peak in spectra of cyclohexylamido, morpholino, diethylamido, pyrrolidino, piperidino, and nonylamido compounds; but m/e 198 is the base peak for both dimethylamido and isopropylamido compounds.

(ix) From the ^1H NMR spectral studies of the 2-alkoxy/alkylamido compounds it is fairly evident that the chemical shift difference of the two gesinal protons H_{4A} and H_{4B} (two protons of the CH_2 group in the dioxaphosphorin ring) is increasing in going from the methoxy (BD-8) to the cyclohexylamido (BD-10) compounds. 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 (also in other phosphoramidothionates), this may be mainly in one conformation (conformation II, p- 160), 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 the 5.2 ppm proton (J = 25.6 H_z) and the quasi-axial proton H_{4A} is assigned to the 5.6 ppm. proton (J = 6.3 Hz); the geminal coupling constant is 14.7 Hz. It has also been observed that the proton H_{4B} is coupled equally to the three aromatic protons H_5, H_7 and H_8 with J = 0.6. Hz; the proton H_{4A} is more strongly coupled to H_5 and H_7 with J = 1.0 Hz, but not to H_8.

From the temperature dependent ^1H NMR spectral study at 270 MHz in the temperature range -70°C to +50°C of the methoxy compound (BD-8), it is fairly evident that the methylene protons of the hetero ring are not equivalent to each other, and the dioxaphosphorin ring is conformationally mobile in solution.

From the ^{13}C NMR spectral study of the pyrrolidino and piperidino compounds (BD-15 and BD-16) it has been observed that the coupling (due to ^{31}P) to the CH_2 carbon (C_4) in the dioxaphosphorin ring changes only from 5.53 Hz in BD-15 to 5.75 Hz in BD-16. This probably means that the conformation is almost the same, and this is in accord with the small difference in ^{13}C chemical shifts, 66.11 and 65.52 ppm. The change in δ (^{13}C) is only 0.6 ppm for the carbon (C_4), and since ^{13}C chemical shifts are often quite sensitive to conformation, it supports the similarity of the

structures.

From ^{31}P NMR spectral studies it is fairly evident that the compounds (BD-15 and BD-16) are stable in one conformation (structure II).

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

(x) The biological activities and other data justify further examination of these phosphorothionates and other related compounds as potential pesticides.

TABLES AND REFERENCES

Table-5A

Acetylcholinesterase Inhibition in Housefly-head homogenate (HFAChE) at 30°C of ED-10.

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

Sets	Inhibitor* Conc. (μg)	O.D.	$\triangle$ O.D.	%Inhibition (Y)	I_{50} (M)
Control	-	0.67	0	-	
1	60	0.26	0.32	56.13	
2	50	0.28	0.29	50.87	
3	40	0.32	0.25	43.85	2.8611×10^{-5}
4	30	0.35	0.22	38.60	
5	20	0.405	0.165	28.90	

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

Regression constants:

Y = mx + c

c = -44.5645

m = 56.1474

r = .9963

Table - 5B

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

Sets	Inhibitor* Conc.(g)	O.D.	ΔO.D.	%Inhibition (Y)	I_{50} (M)
Control	(-)	0.510	0	-	
1	50	0.310	0.200	39.21	
2	40	0.340	0.170	33.35	
3	30	0.370	0.140	27.45	5.4326×10^{-5}
4	20	0.415	0.096	18.60	
5	10	0.480	0.030	5.88	

X* = log [Cond. of the inhibitor in g]

Regression Constants: Y = mx + c

c = - 42.0753

m = 47.3029

r = 0.9983

Table-5C

Acetylcholinesterase Inhibition in Housefly-head homogenate (HFAChE) at 30°C of BD-12

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

Sets	Inhibition* Conc.(μg)	O.D.	$\triangle$O.D.	%Inhibition (Y)	I_{50} (M)
Control	(-)	1.05	0	-	
1	100	0.57	0.48	45.71	
2	80	0.59	0.46	43.81	
3	60	0.64	0.41	39.09	8.2601×10^{-5}
4	40	0.71	0.34	32.38	
5	20	0.80	0.25	23.81	
6	10	0.92	0.13	12.38	

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

Regression constants : Y = mx + c

c = -20.6648

m = 33.5095

r = .9986

Table - 8D

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

(Phosphate buffer, pH 8.0; total volume 5.15 ml/5 fly head; λ =626; incubation time 30 min.)

Sets	Inhibition* Conc. (μg)	O.D.	Δ O.D.	% Inhibition (Y)	I_{50} (M)
Control	(-)	1.05	0	-	
1	100	0.21	0.84	80.00	
2	80	0.27	0.78	74.28	1.80×10^{-6}
3	60	0.33	0.72	68.57	
4	40	0.42	0.63	60.00	
5	20	0.58	0.47	44.76	

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

Regression constants : Y = mx + c

c = -20.0339

m = 49.8328

r = 0.9997

Table - 68

Acetylcholinesterase Inhibition in Housefly-head homogenate (HFAChE) at 30°C of BD-14

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

Sets	Inhibition* Conc. (μg)	O.D.	Δ O.D.	%Inhibition (Y)	I_{50} (M)
Control	(-)	1.06	0	-	
1	50	0.41	0.64	60.95	
2	40	0.46	0.59	56.18	
3	30	0.51	0.54	51.42	1.8642×10^{-5}
4	20	0.59	0.46	43.81	
5	10	0.71	0.36	32.38	

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

Regression constants : Y = mx + c

c = -8.5553

m = 40.6143

r = 0.9999

Table - 6A

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

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

Sets	Inhibitor* Conc.(µg)	O.D.	Δ O.D.	%Inhibition (Y)	I_{50} (M)
Control	(-)	0.69	-		
1	30	0.63	0.06	8.69	
2	40	0.60	0.09	13.04	
3	60	0.56	0.13	18.84	
4	80	0.515	0.175	25.36	1.810×10^{-4}
5	100	0.48	0.21	30.43	
6	120	0.46	0.23	33.33	

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

Regression constants: $Y = mx + c$

$c = -53.8102$

$m = 41.7679$

$r = 0.9969$

Table - 6B

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

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

Sets	Inhibitor* Conc. (μg)	O.D.	Δ O.D.	%Inhibition (Y)	I_{50} (M)
Control	-	0.80	-		
1	30	0.69	0.11	13.75	
2	40	0.65	0.15	18.75	
3	60	0.58	0.22	27.50	
4	80	0.54	0.26	32.50	1.0426×10^{-4}
5	100	0.49	0.31	38.75	
6	120	0.45	0.35	43.75	

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

Regression constants:

Y = mx + c

c = -59.8146

m = 49.2530

r = 0.9968

Table - 6C

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

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

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

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

Regression constants:

$y = mx + c$

$c = -78.8637$

$m = 54.9311$

$r = 0.9995$

Table - 6D

Acetylcholinesterase Inhibition in goat whole-blood of BD-15 at 30°C.

(Phosphate buffer, pH = 8.0; total volume 5.15 ml/0.2 ml blood; λ = 626 nm; incubation time 30 min.)

Sets	Inhibitor* Conc. (μg)	O.D.	$\triangle$ O.D.	%Inhibition (Y)	I_{50} (M)
Control	-	0.69	-		
1	30	0.65	0.04	6.15	
2	40	0.62	0.07	10.76	
3	60	0.58	0.11	15.94	
4	80	0.55	0.14	20.28	4.6564×10^{-4}
5	100	0.53	0.16	23.18	
6	120	0.51	0.18	26.08	

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

Regression Constants:

Y = mx + c

c = -41.7889

m = 32.5769

r = 0.9996

Table -63

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

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

Sets	Inhibitor* Conc. (μg)	O.D.	$\triangle$ O.D.	%Inhibition (Y)	I_{50} (M)
Control	-	0.55	-		
1	50	0.47	0.08	14.54	
2	100	0.42	0.13	23.63	
3	150	0.39	0.16	29.09	4.7805×10^{-4}
4	200	0.37	0.18	32.72	
5	250	0.35	0.20	36.36	

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

Regression constants; $Y = mx + c$

$c = -38.0605$

$m = 30.8911$

$r = 0.9936$

Table - 7a

Chemical hydrolysis of BD-10 at λ=400 nm pH 11.85, 0.0095 M NaOH (in 50% Ethanol) ϵ=19090, Initial concentration, $C_o = 3.0 \times 10^{-5}$, temperature 20°C.

time (min) (X)	O.D.	$C_t \times 10^5$ (M)	$\log \frac{C_o}{C_o - C_t}$ (Y)	K_{hyd} (min^{-1})
5	0.04	0.20953	0.0314	
10	0.07	0.36668	0.0566	
15	0.092	0.48193	0.0760	
20	0.120	0.62860	0.1021	10.824×10^{-3}
25	0.142	0.74384	0.1237	
30	0.163	0.85385	0.1455	
35	0.184	0.96386	0.1683	
40.5	0.207	1.0843	0.1948	
45	0.223	1.1682	0.2142	
50.25	0.244	1.2782	0.2411	
54.75	0.259	1.3567	0.2614	
60	0.276	1.4458	0.2856	

Regression constants: $y = mx + c$

$c = 0.0085$

$m = 0.0047$

$r = 0.9999$

Table - 7b

Chemical hydrolysis of BD-10 at λ = 410 nm pH 11.85, 0.0096 N NaOH (in 50% Ethanol) ϵ =20090, Initial concentration $C_o = 3.0 \times 10^{-5}$ M, temperature 20°C.

Time (min) (X)	O. D.	$C_t \times 10^5$ (M)	$\log \frac{C_o}{C_o - C_t}$ (Y)	K_{hyd} (min^{-1})
5.5	0.047	0.23375	0.0363	
10.5	0.077	0.38328	0.0594	
15.5	0.103	0.51269	0.0814	
20.5	0.127	0.63216	0.1028	
25.5	0.150	0.74664	0.1243	10.364×10^{-3}
30.5	0.173	0.86112	0.1469	
35.5	0.195	0.97063	0.1698	
41.25	0.216	1.0752	0.1927	
45.75	0.236	1.1747	0.2168	
53.5	0.267	1.3290	0.2541	
55.25	0.273	1.3589	0.2620	
60.5	0.292	1.4535	0.2878	

Regression constants : $y = mx + c$

$c = 0.0096$

$m = 0.0045$

$r = 0.9997$

Table - 7a

Chemical hydrolysis of ED-10 at λ =420 nm pH 11.85, 0.0095 M NaOH (in 50% Ethanol) ϵ =18180, Initial concentration C_o = 3.0 x 10^{-5} M temperature 20°C.

time (min) (X)	O.D.	$C_t \times 10^5$ (M)	$\log \frac{C_o}{C_o - C_t}$ (Y)	K_{hyd} (min^{-1})
6	0.047	0.25853	0.0391	
11	0.072	0.39604	0.0615	
16.25	0.096	0.52805	0.0841	
21	0.117	0.64356	0.1049	
26	0.137	0.75358	0.1256	
31	0.157	0.86359	0.1474	10.364 x 10^{-3}
36	0.177	0.97360	0.1704	
41.75	0.197	1.0836	0.1946	
46.5	0.214	1.1771	0.2164	
51.75	0.234	1.2871	0.2434	
56	0.248	1.3641	0.2634	
61	0.264	1.4521	0.2874	

Regression constants:

$y = mx + c$

$c = 0.0104$

$m = 0.0045$

$r = 0.9997$

Table - 7d

Chemical hydrolysis of BD-10 at λ =430 nm pH 11.88, 0.0095 M NaOH (in 50% Ethanol) ϵ =14460, Initial concentration, $C_o = 3.0 \times 10^{-5}$ M, temperature 20°C.

time (min) (X)	O.D.	$C_t \times 10^5$ (M)	$\log \frac{C_o}{C_o - C_t}$ (Y)	K_{hyd} (min^{-1})
7.25	0.042	0.29066	0.0443	
11.75	0.06	0.41522	0.0647	
16.75	0.077	0.53287	0.0849	
21.75	0.094	0.65052	0.1061	
26.5	0.108	0.74740	0.1244	
31.5	0.125	0.86506	0.1477	9.903×10^{-3}
36.5	0.139	0.96194	0.1679	
42.5	0.156	1.0727	0.1922	
47.25	0.168	1.1626	0.2129	
52.75	0.185	1.2803	0.2417	
56.5	0.193	1.3356	0.2659	
61.5	0.205	1.4187	0.2781	

Regression constants:

$y = mx + c$

$c = 0.0125$

$m = 0.0043$

$r = 0.9997$

Table - 8a

Chemical hydrolysis of BD-11 at λ =400 nm pH 11.85, 0.0096 M NaOH (in 50% Ethanol) ϵ =19000, Initial concentration, C_o = 7.06 x 10^{-5} M temperature 20°C.

time (hrs) (X)	O.D.	$C_t \times 10^5$ (M)	$\log \frac{C_o}{C_o - (X) C_t}$	K_{hyd} (min^{-1})
5	0.098	0.51336	0.0328	
27.75	0.303	1.5872	0.1106	
52.5	0.423	2.2158	0.1636	6.1413×10^{-5}
75.33	0.46	2.4086	0.1813	
124.6	0.56	2.9335	0.2332	

Regression constants :

$y = mx + c$

$c = 0.0544$

$m = 0.0016$

$r = 0.9536$

Table - 8b

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

time (hrs) (X)	O.D.	$C_t \times 10^5$ (M)	$\log \frac{C_o}{C_o - C_t}$ (Y)	K_{hyd} (min^{-1})
5	0.103	0.51269	0.0327	
27.75	0.316	1.5729	0.1095	
52.5	0.446	2.2150	0.1635	
75.33	0.49	2.4390	0.1841	6.1413×10^{-5}
124.6	0.6	2.9866	0.2388	

Regression constants :

$y = mx + c$

$c = 0.0526$

$m = 0.0016$

$r = 0.9599$

Table - 8c

Chemical hydrolysis of BD-11 at λ =420 nm pH 11.85, 0.0095 M NaOH (in 50% Ethanol) ϵ =18180, Initial concentration, $C_o = 7.06 \times 10^{-5}$ M, temperature 20°C.

time (hrs) (X)	O.D.	$C_t \times 10^5$ (M)	$\log \frac{C_o}{C_o - C_t}$ (Y)	K_{hyd} (min^{-1})
6	0.093	0.51155	0.0327	
27.75	0.284	1.5622	0.1086	
52.5	0.400	2.2002	0.1622	6.1413×10^{-5}
75.33	0.45	2.4752	0.1876	
124.6	0.54	2.9703	0.2371	

Regression constants:

$y = mx + c$

$c = 0.0527$

$m = 0.0016$

$r = 0.9589$

Table - 8d

Chemical hydrolysis of ED-11 at λ=430 nm pH 11.85, 0.0096 M NaOH (in 50% Ethanol) ϵ=14450, Initial concentration, $C_o = 7.06 \times 10^{-5}$ M, temperature 20°C.

time (hrs) (X)	O.D.	$C_t \times 10^5$ (M)	$\log \frac{C_o}{C_o - C_t}$ (Y)	K_{hyd} (min^{-1})
5	0.074	0.61211	0.0327	
27.75	0.220	1.5825	0.1055	
52.5	0.315	2.1799	0.1604	6.1413×10^{-5}
75.33	0.355	2.4567	0.1357	
124.6	0.428	2.9619	0.2362	

Regression constants: $y = mx + c$

$c = 0.0511$

$m = 0.0016$

$r = 0.9620$

Table - 9a

Chemical hydrolysis of BD-13 at λ=400 nm pH 11.85, 0.0095 M NaOH (in 50% Ethanol) ϵ =19000, Initial concentration, $C_o = 7.3 \times 10^{-5}$ M, temperature 20°c

time (hrs) (X)	O.D.	$C_t \times 10^5$ (M)	$\log \frac{C_o}{C_o - C_t}$ (Y)	K_{hyd} (min^{-1})
5	0.032	0.15763	0.0101	
28	0.138	0.72289	0.0463	
52.75	0.203	1.0634	0.0684	2.687×10^{-5}
75.25	0.24	1.2572	0.0821	
124.75	0.293	1.5348	0.1025	

Regression constants:

$y = mx + c$

$c = 0.0195$

$m = 0.0007$

$r = 0.9966$

Table-9b

Chemical hydrolysis of BD-13 at λ =410 nm pH 11.86, 0.0095 M NaOH (in 50% Ethanol) ϵ =20090, Initial concentration, $C_o = 7.3 \times 10^{-5}$, temperature 20°C.

time (hrs) (X)	O.D.	$C_t \times 10^5$ (M)	$\log \frac{C_o}{C_o - C_t}$ (Y)	K_{hyd} (min^{-1})
5	0.034	0.16924	0.0102	
28	0.143	0.71180	0.0446	2.687×10^{-5}
52.75	0.210	1.0453	0.0671	
75.25	0.255	1.2693	0.0830	
124.75	0.305	1.5182	0.1013	

Regression constants:

$y = mx + c$

$c = 0.0193$

$m = 0.0007$

$r = 0.9859$

Table - 9a

Chemical hydrolysis of BD-13 at λ=420 nm pH 11.85, 0.0095 M NaOH (In 50% Ethanol) ϵ=18180, Initial concentration, C_o = 7.3 x 10^{-5} M, temperature 20°C.

time (hrs) (X)	O.D.	$C_t \times 10^5$ (M)	$\log \frac{C_o}{C_o - C_t}$ (Y)	K_{hyd} (min^{-1})
5	0.028	0.15402	0.0093	
28	0.129	0.70957	0.0444	3.07×10^{-5}
52.75	0.183	1.0616	0.0682	
75.25	0.23	1.2651	0.0827	
124.75	0.28	1.5402	0.1029	

Regression constants:

$y = mx + c$

$c = 0.0117$

$m = 0.0008$

$r = 0.9600$

Table- 9d

Chemical hydrolysis of ED-13 at λ = 430 nm pH 11.85, 0.0095 M NaOH (in 50% Ethanol) ϵ =14450, Initial concentration, $C_o = 7.3 \times 10^{-5}$ M, temperature 20°C.

time (hrs) (X)	O.D.	$C_t \times 10^5$ (M)	$\log \frac{C_o}{C_o - C_t}$ (Y)	K_{hyd} (min^{-1})
5	0.022	0.15325	0.0092	
28	0.100	0.69204	0.0433	
52.75	0.147	1.0173	0.0652	3.07×10^{-5}
75.25	0.18	1.2457	0.0813	
124.75	0.221	1.5234	0.1021	

Regression constants:

$y = mx + c$

$c = 0.0110$

$m = 0.0008$

$r = 0.9648$

Table - 10n

Chemical hydrolysis of BD-17 at λ=400 nm pH 11.85, 0.0095 M NaOH (1n 60% Ethanol) ϵ =19090, Initial concentration, $C_o = 3.14 \times 10^{-5}$ M, temperature 20°C.

time (min) (X)	O.D.	$C_t \times 10^5$ (M)	$\log \frac{C_o}{C_o - C_t}$ (Y)	K_{hyd} (min^{-1})
4.6	0.072	0.37716	0.0556	
8.08 (8min 5Secs)	0.110	0.57622	0.0830	
10	0.130	0.68093	0.1062	
12	0.150	0.78573	0.1251	
16	0.180	0.94290	0.1551	20.266×10^{-3}
20	0.220	1.1524	0.1986	
23	0.240	1.2572	0.221	
26	0.262	1.3724	0.2405	
29	0.282	1.4772	0.2761	
35	0.316	1.6553	0.3259	
40	0.342	1.7915	0.3671	
45	0.368	1.9277	0.4133	

Regression constants:

$y = mx + c$

$c = 0.0194$

$m = 0.0038$

$r = 0.9997$

Table-10b

Chemical hydrolysis of BD-17 at λ=410 nm pH 11.85, 0.0095 M NaOH (in 50% Ethanol) ϵ =20090, Initial concentration, C_o = 3.14 x 10^{-5} M, temperature 20°C.

time (min) (X)	O.D.	C_t x 10^5 (M)	$\log \frac{C_o}{C_o - C_t}$ (Y)	K_{hyd} (min^{-1})
5.25	0.07	0.34843	0.0511	
6	0.077	0.38323	0.0563	
10	0.127	0.63216	0.0976	
15	0.177	0.88104	0.1430	
20	0.227	1.085	0.1852	20.266 x 10^{-3}
25	0.265	1.3191	0.2382	
30	0.299	1.4783	0.2764	
35	0.333	1.6575	0.3259	
40	0.355	1.7670	0.3503	
45	0.373	1.8815	0.3971	

Regression constants:

$y = mx + c$

$c = 0.0083$

$m = 0.0088$

$r = 0.9911$

Table-10c

Chemical hydrolysis of BD-17 at λ=420 nm pH 11.85, 0.0095 M NaOH (in 60% Ethanol) ϵ = 18180, Initial concentration, $C_o = 3.14 \times 10^{-5}$ M, temperature 20°C.

time (min) (X)	O.D.	$C_t \times 10^5$ (M)	$\log \frac{C_o}{C_o - C_t}$ (Y)	K_{hyd} (min^{-1})
11	0.121	0.66557	0.1035	
16	0.168	0.92409	0.1514	
21	0.206	1.1276	0.1932	
26	0.237	1.3036	0.2330	19.345×10^{-3}
31	0.270	1.4861	0.2782	
36	0.295	1.6474	0.3230	
41	0.320	1.7602	0.3671	
46	0.342	1.8812	0.3970	

Regression constants:

$y = mx + c$

$c = 0.0152$

$m = 0.0084$

$r = 0.9994$

Table-10d

Chemical hydrolysis of BD-17 at λ=430 nm pH 11.85, 0.0095 M NaOH (in 50% Ethanol) ϵ = 14460, Initial concentration, C_o = 3.14 x 10^{-5}M, temperature 20°C.

time (min) (X)	O.D.	$C_t \times 10^5$ (M)	$\log \frac{C_o}{C_o - C_t}$ (Y)	K_{hyd} (min^{-1})
7.16 (7 min 10 secs)	0.063	0.43599	0.0649	
12	0.100	0.69204	0.1081	
17	0.130	0.89965	0.1466	
22	0.160	1.1073	0.1889	17.733×10^{-3}
27	0.186	1.2872	0.2291	
32	0.208	1.4394	0.2663	
37	0.228	1.5779	0.3032	
42	0.245	1.6955	0.3372	
47	0.260	1.7993	0.3696	

Regression constants:

$y = mx + c$

$c = 0.0165$

$m = 0.0077$

$r = 0.9990$

Table - 11a*

Chemical hydrolysis of BD-15 pH 11.85, 0.0095 N NaOH (in 50% Ethanol), Initial concentration, C_o = 2.12 x 10^{-5} M, temperature 20°C.

λ (nm)	time (hrs) (X)	O.D.	$C_t \times 10^5$	$\log \frac{C_o}{C_o - C_t}$ (Y)	K_{hyd} (min^{-1})
400	150	0.06	0.31430	0.0697	1.7835 x 10^{-5}
410	150	0.063	0.31359	0.0695	1.7784 x 10^{-5}
420	150	0.056	0.30803	0.0682	1.7462 x 10^{-5}
430	150	0.045	0.31142	0.0690	1.7456 x 10^{-5}

* This compound (BD-15) was extremely resistant to hydrolysis at pH 11.85; only after 150 hours readings were taken at λ =400, 410, 420 and 430 nm and K_{hyd} was calculated from these readings with the help of 1st order rate equation

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

Table - 11b*

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

λ (nm)	time (hrs) (X)	O.D.	$C_t \times 10^5$ (M)	$\log \frac{C_o}{C_o - C_t}$ (Y)	K_{hyd} (min^{-1})
400	150	0.038	0.19906	0.0428	1.0952×10^{-5}
410	150	0.044	0.21901	0.0474	1.2129×10^{-5}
420	150	0.038	0.20902	0.0451	1.1541×10^{-5}
430	150	0.031	0.21453	0.0463	1.1856×10^{-5}

*This compound (BD-16) also is extremely resistant to hydrolysis at pH 11.85; only after 150 hours readings were taken at λ=400, 410, 420 and 430 nm and K_{hyd} was calculated from these readings with the help of 1st order rate equation: $K = \frac{1}{t} \ln \frac{C_o}{C_o - C_t}$

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