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Synthesis and Spectral Identification of Novel Alkyl-Aryl Phosphoric Amide-An Antibacterial Agent

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ABSTRACT: Synthetic O-Methyl,N-2-Cl,4-NO_2-Phenylphosphoric amide, a mixed disaster, containing one C-N-P and C-O-P linkage together in it, has been tested for its antibacterial activity. The compound has been studied against Gram –ve bacterium, *E. coli*, in DMSO medium while using the test compound, as required. The results obtained depict that the test compound exhibits a significant antibacterial activity. The diester, has been formed by a two-step process and has been identified by employing IR, ^{1}HNMR and ^{13}C NMR spectral techniques.

Keywords: Phosphorochloride, Phosphoranidate, Antibacterial

I. INTRODUCTION

Phosphate compounds are extraordinarily important type of compounds, being involved in nucleic acid synthesis, cellular energetic, gene expression and regulation of metabolism. Phosphate diester linkages are present in ribose nucleic acid that play a significant role in biology (Iyer *et al,* 2008) [1]. Organphosphoramidates have been found to have strong biological importance. Apart from the importance of phosphate compounds, nitro compounds too have a very significant or key role because of their powerful antibiotic and other pharmacological activities. The common broad spectrum antibiotic, chloramphenicol, was the first natural product recognized to possess an aromatic nitro group, and is used for the treatment of typhoid, bacterial meningitis as well as other pencillin resistant staphylococcal infections. Another NO_2 group containing compound, 2-nitro imidazole (Azomicin) has low toxicity and possesses a broad spectrum activity against both bacteria and protozoa. The deciding factors which affect the therapeutic utility of a drug are chemical and physical properties which may be largely unrelated to the presence of nitro or nitroso groups. Therapeutic utility of a drug also depends upon the balance between its medicinal action and detoxification mechanism. Those drugs which are rapidly detoxified may be of limited medicinal use. However the detoxification route may be blocked selectively to enhance the therapeutic effect of a drug. *e.g.* by reduction of nitro substituent's to amino groups.

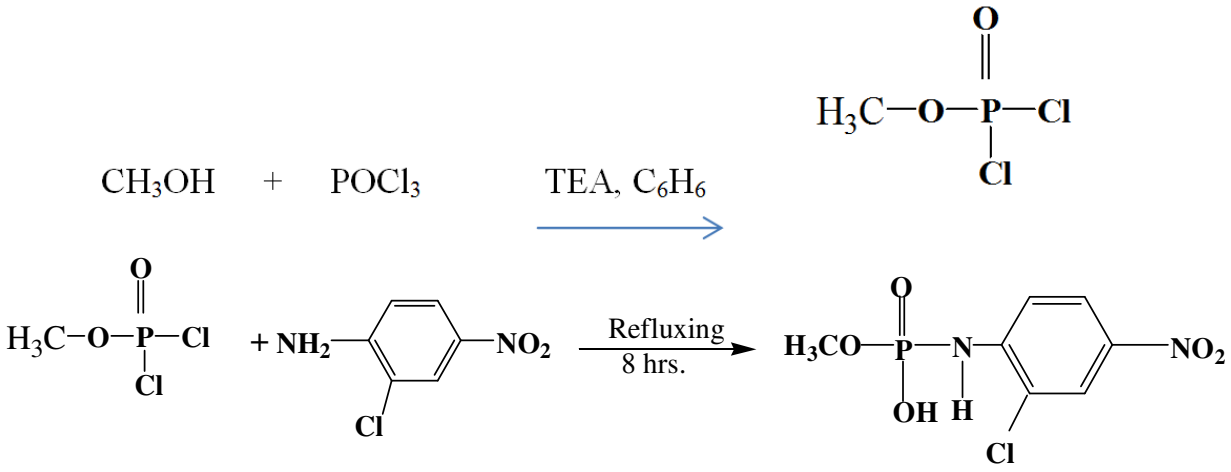
Due to the significance of some of the features related to the presence of the NO_2 group, the present study of O-methyl, N-2-Cl, 4-NO_2-Phenylphosphoric amide has been undertaken. This compound was also expected to exhibit some characteristic behavior of physiological utility. In general majority of the organophosphorous compounds (C-N-P or C-O-P or C-S-P) have biochemical importance because of the role of the orthophosphates (C-O-P) especially as acetylcholinesterase inhibitors or even worse.

II. EXPERIMENTAL

During the present study, $POCl_3$ has been employed for the basic compounds, methanol, β-Naphthol and 2-Cl, 4-NO_2-aniline mainly for the synthesis of the novel compound.

The process of phosphorlylation reaction was carried out in two steps. (I) the first step involved the phosphorlylation of methanol, by the treatment with phosphorus oxy chloride to form the major product as methoxyphosphorodichloridate. In the second step (II), the dichloridate, so formed, was subjected to treatment with 2-Cl- 4-NO_2-aniline to obtain the aimed product. Methanol(0.01mol), T.E.A (0.02mol) and 10.0ml benzene (dry) (Cava and Mitchell, 1969) were taken in a 50mL R.B. flask and $POCL_3$ (0.02mol) was added drop wise to it with shaking and a clear colourless solution was formed. This was subjected to stirring for ten minutes, followed by the addition of second instalment of methanol (0.5mL) and stirring was continued [2].

After 1.35 hrs. of stirring, a reddish-coloured solution was obtained and T.E.A. HCl was seen separating out, thereby, showing the progress of the reaction. Since, the formation of side products was not ruled out, as shown by GC-MS studies (spectrum 1). The reddish-coloured solution formed after refluxing was subjected to TLC and GC-MS analysis.



To the methoxyphosphorodichloridate formed in the first step, 2-Cl,4-NO_2-aniline was directly added in the quantity mentioned above. This did not require any further addition of the solvent, as benzene was present in the dichloridate prepared earlier. The mixture was then transferred to an R. B. flask and with the help of the condenser etc. the refluxing assembly was placed in an oil bath. Heating was carried out for nearly 8.10 hrs. When, the formation of a sticky mass at the bottom of the flask was noticed, the reaction mixture was cooled at r.t. and the upper benzene layer was decanted off. Recrystallisation of the greenish sample with CH_2Cl_2 was carried out to purify the product followed by the tests for the presence of PO_3''' & Cl'. Melting point of the pure compound was found to be 132-135^oC.

III. RESULTS AND DISCUSSION

The synthetic mixed diester may be characterized by the absorption spectroscopic measurements (Gore, 1950; Bellamy and Beecher, 1953; Corbridge, 1956). [3-5]. But before applying these techniques the sample was tested for its purity by TLC and recrystallized with CH_2Cl_2 on the basis of the spot(tic) the purified product was then used for the identification and for the testing its activity against Gram -ve bacteria.

Infrared spectral studies (Colthup, *et al.,* 1964; Bellamy,1975) were made for O-Methyl,N-2-Cl,4-NO_2-Phenylphosphoric amide and the characteristic group frequencies indicate the presence of the P=O group (1281.7cm); C-(Aromatic & -NO_2 (1500.1,1588.4); N-h (3386.6); P-N-Ph (1281.7) and presence of P-N have been indicated. Since the compound is derived from $POCl_3$ the latter itself absorbs at 1300cm^{-1} [6-7].

According to Nyquist, when the –NH group is attached to P=O link, there are two free NH stretching bands (Nyquist, 1963) in dilute solutions. He associated these with the cis and trans forms. This is of course parallel with the behaviour of the secondary amides [8]. Thomas revived (Chittenden and Thomas, 1966) the data regarding the P-N stretching vibrations and suggested that this bond absorbs a very range between 1500cm^{-1} and 870cm^{-1} [9].

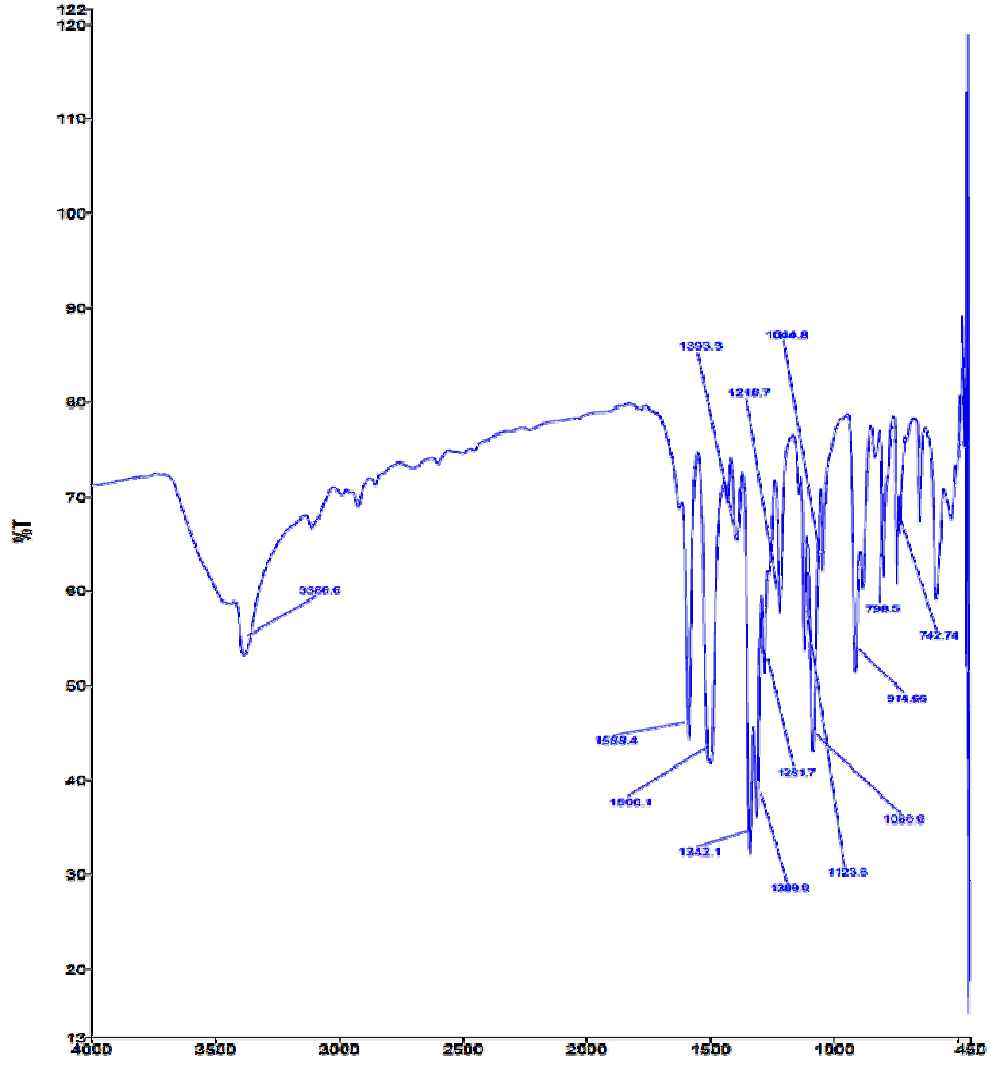
The structure of the Mixed diester-I has also been confirmed with the help of ^1H NMR, ^{13}C NMR and ^{31}P NMR spectral studies. The required type of protons (Silverstein, *et al* 2007) CH_3, CH-O-, P-NH and the aromatic protons are observed at 0.9−1.0 ppm, 3.5-3.9 ppm, 7.9−8.0 ppm respectively. Some other data is also available for 1H-NMR studies and the compound could be examined in DMSO medium [10].

^{13}C NMR spectrum showed the presence of the CH_3−O grouping and the aromatic framework respectively at 50− 55ppm, and 100− 150ppm. This study was also made in DMSO medium.

The most significant aspect of the mixed compound, the presence of the phosphoric amide has been established by the appearance of a sharp signal at 2.325 ppm and for this study 85% H_3PO_4 had been used as standard by the technician. It is already described that such a grouping may lie between a range of δ equal to -25 to +50 ppm.

Interpretation of IR Spectral Data: The IR spectrum(I) gives the presence of the following groups/linkages in the mixed diester-I:

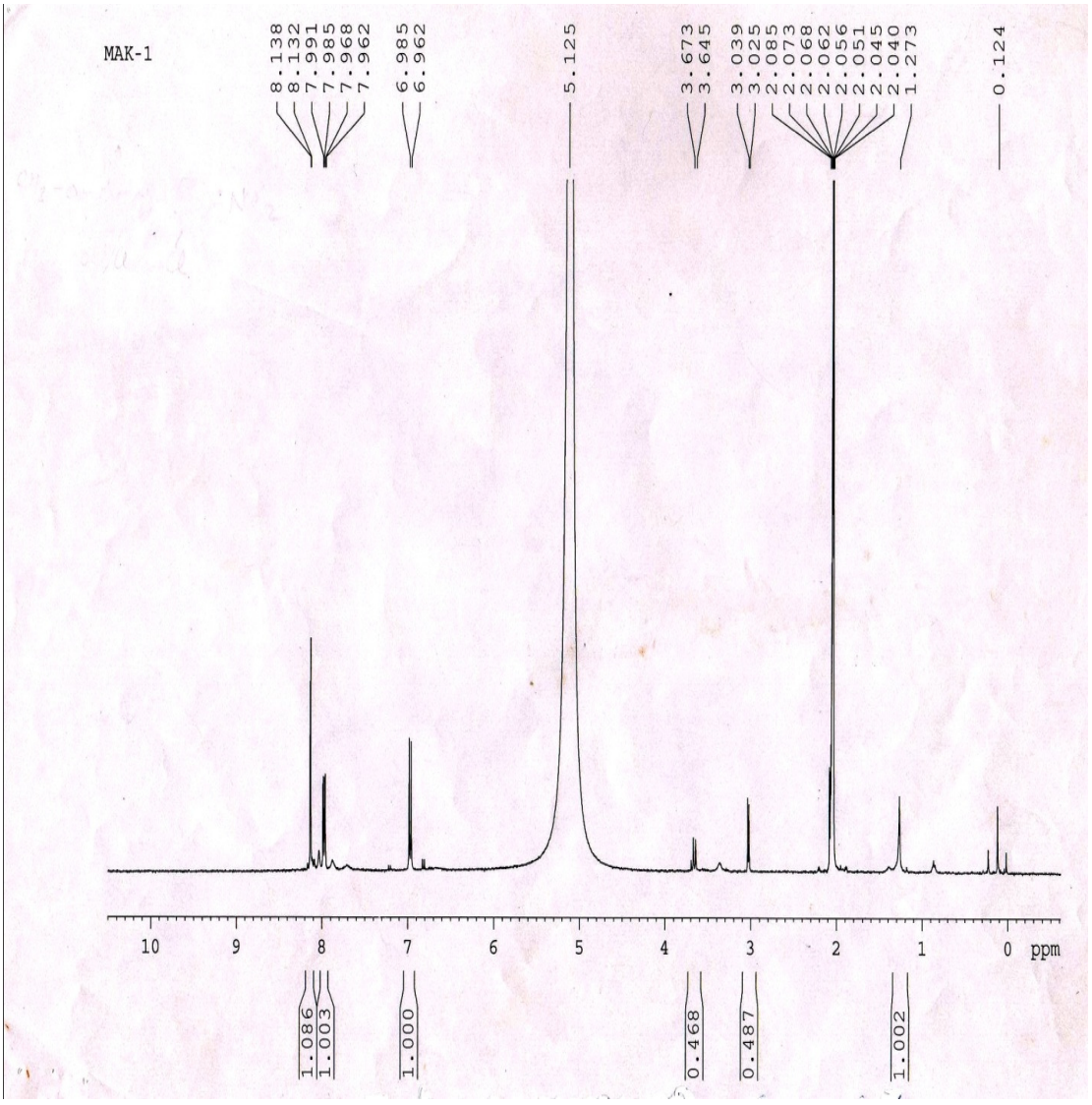
No.	Groups/linkage	ν cm^{-1}(Rep.)	ν cm^{-1}(Obsd.)
(i)	—OH/—NH	37500 – 3000	3386.0
(ii)	P-N-H	3300 – 3100	
(iii)	P-O-H	(a) 2700 – 2500 (b) 2300 – 2100 (c) 1040 – 910	1044
(iv)	P-N-C	ca.1600, 1100 – 800	1588.4, 1086
(v)	P=O	1300 – 1200	1281
(vi)	P-N	1290	1216
(vii)	C-N	(a) 1065 – 950 (b) 910 - 890	914.66
(viii)	P-N	850 – 650	798.5
(ix)	P-O-C	1050 – 970	1044.8
(x)	Aro.-NO_2	1560 – 1515	1588.4, 1500.1
(xi)	C_6H_6 – (p-disubstn.)	840- 810	ca. 810

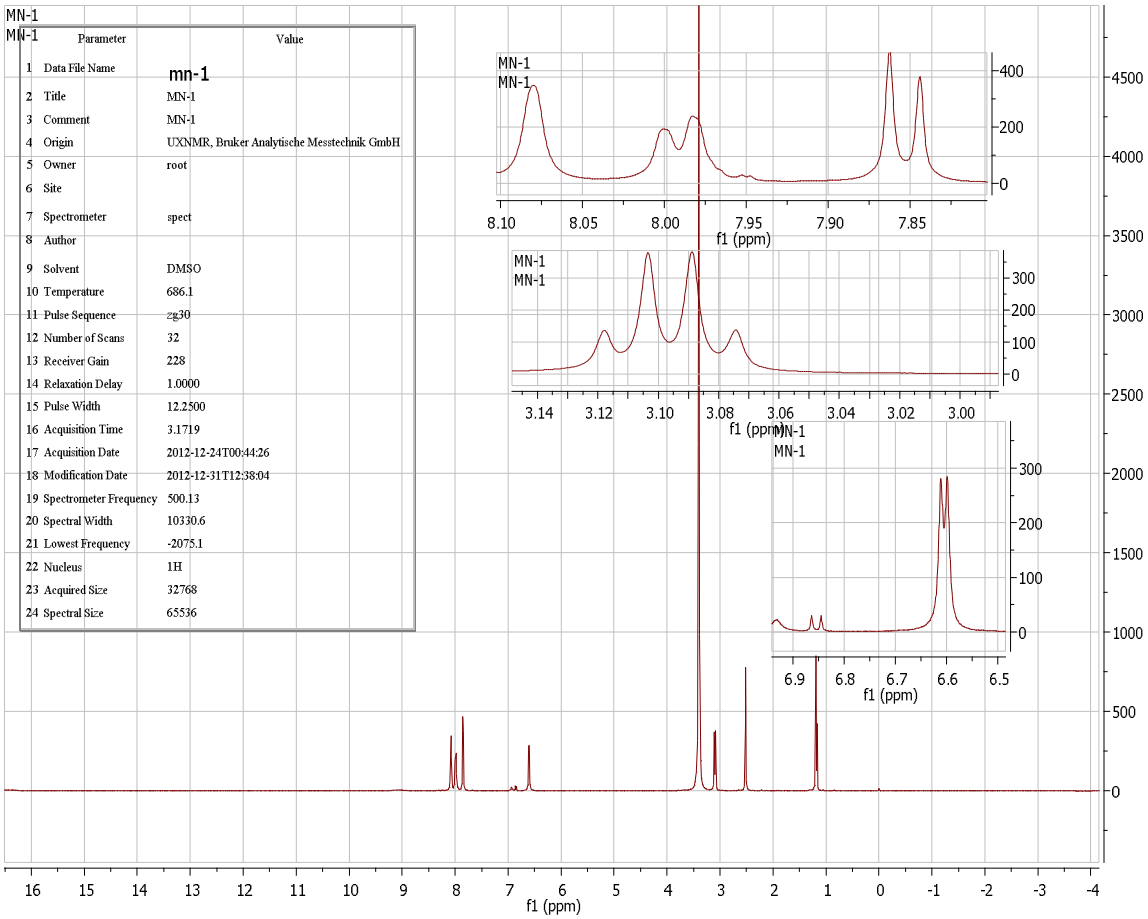


^{1}H NMR Spectral Data by 500 MHz Spectrometer: Spectrum (II) indicates the following types of protons in the molecule, (I):

No.	Linkage	δ (ppm) $_{Rep.}$	δ (ppm) $_{Obsd.}$
(i)	P-NH	7.9 - 8.3	7.95 - 8.00
(ii)	Ar – NH/-OH	(a) 608 – 7.2 (b) 1.5 – 4.0	6.6 (Doublet), 6.85 (Doublet) 3.07 – 3.12 (Quartet)
(iii)	CH_3 –	0.9 – 1.0	0.9 – 1.0
(iv)	Aromatic Protons	7.5 – 8.0	7.85 (Doublet)
(v)	CH_3 – O –	–	3.5-3.9
(vi)	DMSO	2.6	2.5

^{1}HNMR Spectrum: On this basis of this spectrum coupling constants of various protons may be determined on the basis of the values shown on the upper side of the spectrum.



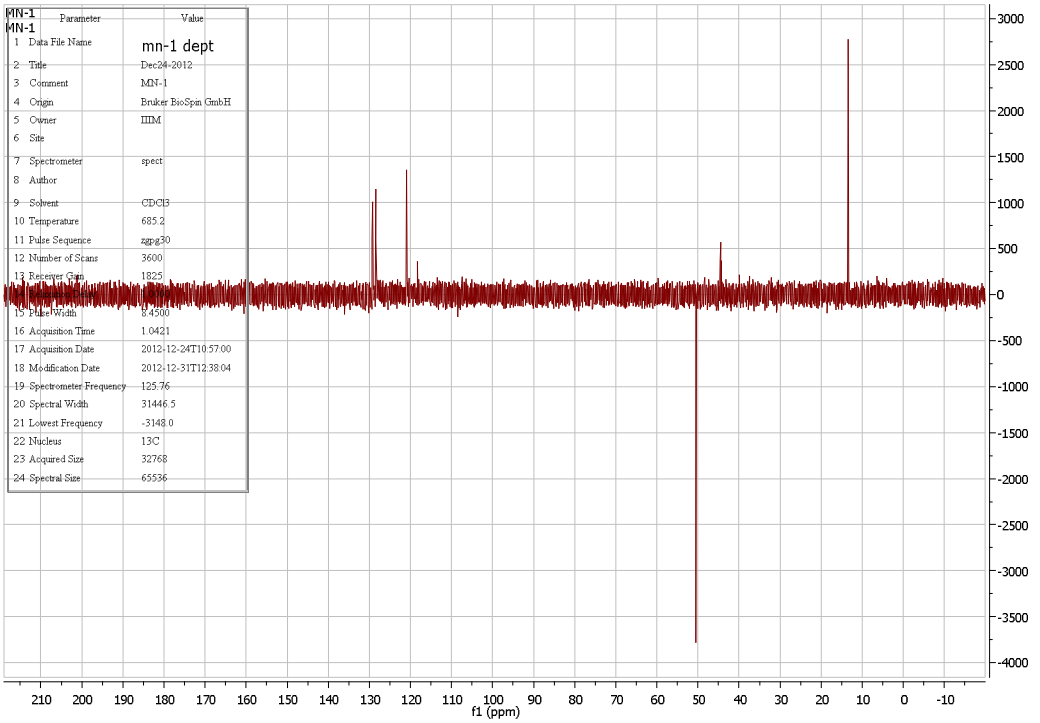


^{1}H-NMR of O-Methyl,N-2-Cl,4-NO_2-Phenylphosphoric amide

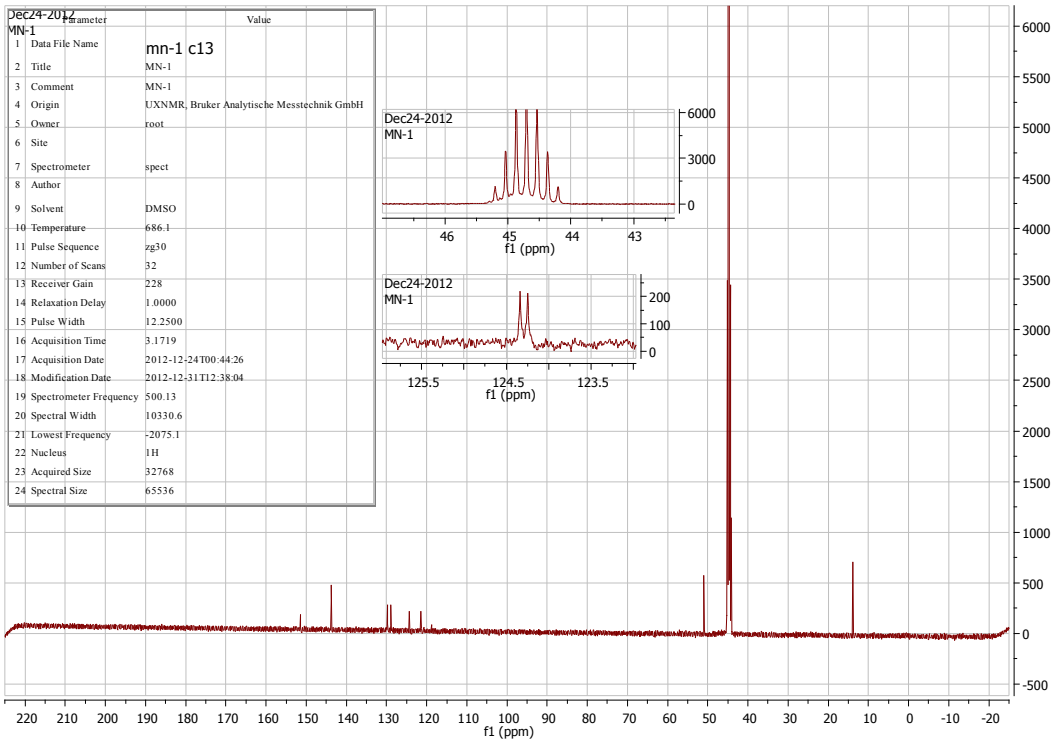
^{13}C NMR Spectral Details (Spectra IV and V):

The ^{13}CNMR Spectrum (IV) and DEPT spectrum (V) have both been recorded for the mixed diester–I with following δ (ppm) values.

Group	δ(ppm) Repod.	δ(ppm) Obsd.
(i) CH_3 – O -	50 – 55	14
(ii) C_6H_6	100 – 150	124.5,130,144
(ii) DMSO	39 – 45	44
DEPT Spectrum:		
(ii) – CH_3	0-80	ca. 13
(ii) Aromatic Carbon	125-150	ca. 121, 129

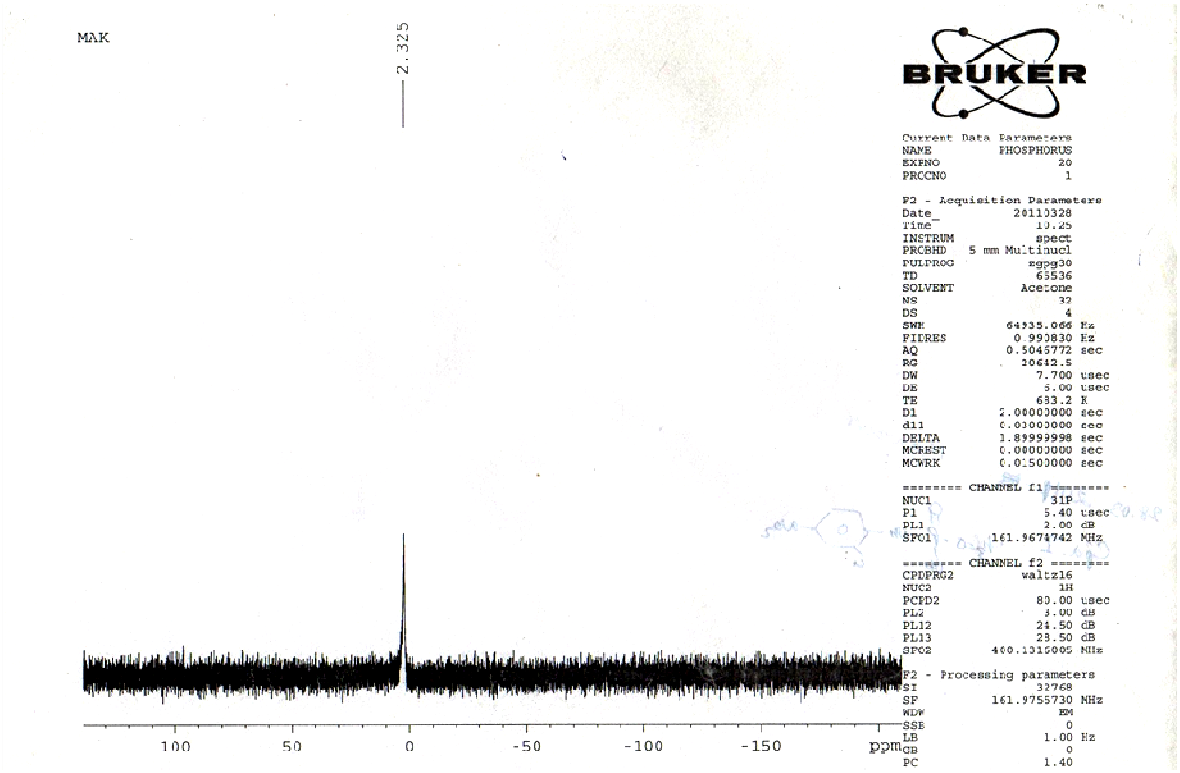


DEPT of O-Methyl,N-2-Cl,4-NO_2-Phenylphosphoric amide



^{13}C NMR-Spectrum of O-Methyl,N-2-Cl,4-NO_2-Phenylphosphoric amide

^{31}P NMR Spectrum (Crutchfield, *et al,* 1967) [11]:

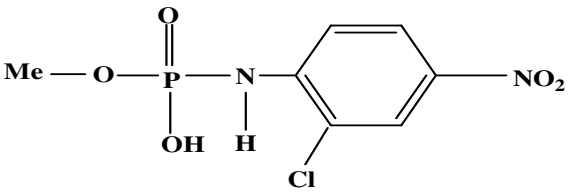


85% - H_3PO_4 has been used as a standard for determining ^{31}PNMR spectrum (VI) of the mixed diester – I.

δ (ppm) $_{Rep.}$	δ (ppm) $_{Obsd.}$
-25 to +50	2.325 ppm

Anti-Microbial Effect: Pathogens, like bacteria, viruses and protozoa are an important source of diseases in man. Proper measures need to be taken to keep the environment relatively clean. In case, this is not so, the pathogens may increase out of proportion and may even cause epidemics. A serious problem today is the development of micro organisms that are resistant to the traditional drugs including antibiotics. Novel drugs are needed to combat the infections/diseases caused by these micro organisms. The present study is thus a step-forward in this direction only. As early as 1944 the bactericidal effect of phenylphosphates was determined (Rosenmund and Hans, 1944) by K.W. Rosenmund and Hansvogt. Only Mono- and Di- Phenyl esters were prepared and tested because triphenyl esters are difficultly soluble and poisonous in nature. An enzyme preparation from *E. coli* itself, has been shown to catalyse (Fujimoto and Smith, 1962) phosphoryl transfer from a number of phosphoric amidates to hexoses [12-13].

It is a matter of great satisfaction that the mixed diesters-I(O-Methyl, N-O-Cl, 4NO_2-phenylphosphoric amide), and mixed diester-II(O-β_Napthyl,N-2-Cl, 4-NO_2-phenylphosphoric amide) shown as below have exhibited significant antibacterial action toward *E. coli* (DH5α) used for the present study.



(I)-Neutral Species

Mixed Diester

Although nitrogen is so widely distributed in nature, its occurrence in the form of nitro group is very limited and natural products containing nitro group are only a few in number. Nevertheless, these compounds have gained importance due to their powerful antibiotic and other pharmacological activity, in which the nitro group plays a key role.

Some of the compounds like chloramphenicol (streptomycesvenezuelae), azomycin (Nocardiamesenterica), respectively show both the antibiotic as well as the antibacterial activities. In the light of these studies, it may also be proposed that the nitro-bearing phenyl system in both the above mixed diesters in responsible for its antibacterial activity against *E. coli* variety mentioned earlier.

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