EFFECTS OF ORGANOPHOSPHORUS COMPOUNDS WITH NON POLAR SIDE CHAINS ON ENZYMES

THESIS

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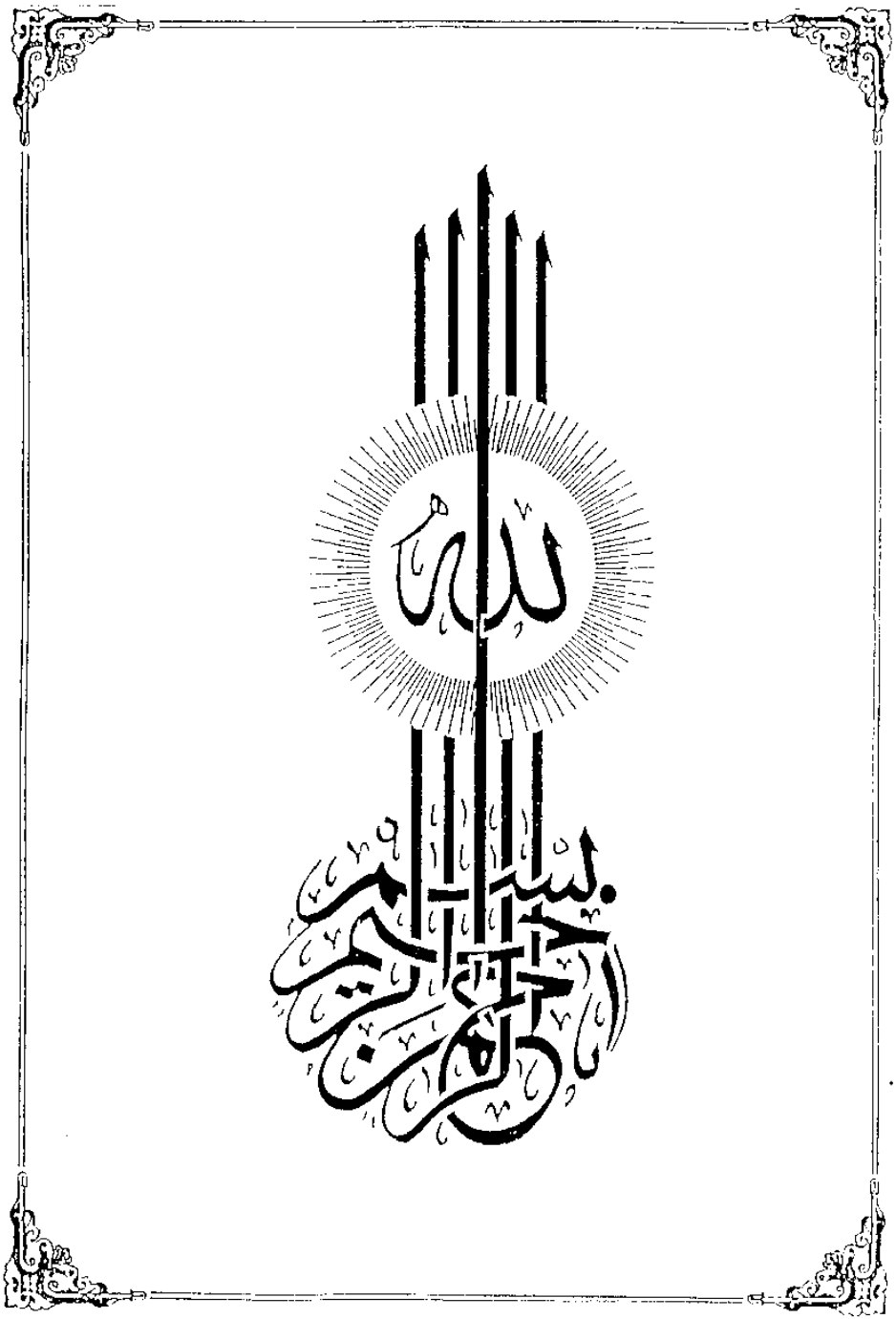

AIM OF THE WORK

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Organophosphorus compounds are considered hazardous to personel subjected to them either during preparation or use as pesticides. They also constitute a pollitant to the environment and could affect human being by being ingested with different plants consumed by people. Many organophosphorus compounds are yearly synthesized to be used for different reasons. In this work, the effect of special group of organophosphorus compounds with non polar aromatic side chain will be tested.

Cholinesterase, lipase and transaminases are the prime concern in this work.

INTRODUCTION

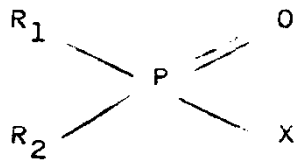
ORGANOPHOSPHORUS COMPOUNDS

The insecticidal properties of these compounds were discovered in Germany during the second World War, when allied and German authorities were engaged in a search for substances suitable for chemical warfare as so called nerve gases.

Whilst several compounds were synthesised and tested for this purpose, they were fortunately never used.

Numerous organophosphorus compounds with widely different chemical structure are now available. An essential feature of these compounds is the variety of side chains that can be attached to one or more parts of the component nucleus (Wilson et al., 1975).

Chemistry:

The general formula for this class of cholinesterase inhibitors is  (Schrader, 1952).

A great variety of substituents is possible: R_1 and R_2 may be alkyl, alkoxy, aryloxy, amido, mercaptan or other groups and X may represent a halide, cyanide, thiocyanate, phenoxy, thiophenoxy, phosphate or carboxylate group.

Classification:

Group A which includes, DFP (diisopropylfluorophosphate) Mipafox, Sarin, Soman and Tabun.

Where X is a halogen except the latter which is cyanide. The last three are termed nerve gases.

- Group B which includes for example paroxon the active metabolite of parathion where X is alkyl or alkoxy or aryloxy group.
- Group C which includes parathion, Malathion and ethyl 4-nitrophenyl phenylphosphate (EPN).

  Those are thiol or thionophosphorus compounds.
- Group D which includes Tetrarethyl pyrophosphate (TEPP) and octamethyl pyrophosphoramide (OMPA) containing pyrophosphate.

- Group E e.g. echothiophate which contained quaternary ammonium group (Holmstedt, 1959, 1963).

Organophosphorus compounds are powerful inhibitors of cholinesterase and are effective against a wide range of insects and pests.

They can be formulated as dusts and granules for soil application, as aqueous solutions or suspensions for spraying crops and buildings and as sheep dips, impregenated strips of plastic, from which the active compound is slowly released, are used indoors to eliminate house-hold flies and other nuisance insects.

Toxic effects:

Poisoning by these compounds occurs amongst agricultural workers. These insecticides are absorbed through the skin, conjunctiva and alimentary tract or by inhalation.

The onset of poisoning is insidious and is characterised by anorexia, nausea, excessive sweating and salivation. In more severe cases there is constriction

of the pupils, pulmonary oedema and muscular twitching first of the eye lids and later of most voluntary muscles, death occurs from neuromuscular paralysis (Wilson et al., 1975).

Delayed effects of organophosphates exposure:

Persons exposed to organophosphorus insecticides for a long period and who did not develop acute manifestations show some delayed effects such as delayed peripheral neuropathy (Baron, 1981).

Biochemical mechanism of delayed neurotoxicity:

Since all organophosphate esters inducing a delayed neuropathy are either direct esterase inhibitors or are metabolically, converted to inhibitors, it has been generally considered that phosphorylation of an esterase although not necessarily in the nervous system is probably an essential prerequisite to the onset of delayed neuropathy (Baron, 1981).

Inhibition of specific protein which was referred to as neurotoxic esterase (NTE) having esteratic activity

was associated with delayed neurotoxicity (Aldridge and Barnes, 1961, 1966) & (Barnes and Denz, 1953) & (Aldridge et al., 1969) & (Clothier and Johnson, 1979) & (Johnson, 1969) & (Johnson and Barnes, 1970) & (Lotti and Johnson, 1968, 1979).

It was suggested that approximately 70-80% of NTE activity must be phosphorylated through an irreversible or aged reaction in order for the chemical in question to be considered as a delayed neurotoxicant (Johnson, 1969, 1974, 1977).

NTE has been shown to occur in both neural and non neural tissue from mammalian and avian species (Johnson, 1969) & (Lotti and Johnson, 1978, 1979).

Organophosphates act by phosphorylation of esterase enzymes giving rise to irreversibly or slowly reversibly inhibited enzymes.

Spontaneous hydrolysis of phosphorylated enzyme leading to a reactivated protein is counteracted by spontaneous reaction termed aging which leads to stable

irreversibly phosphorylated enzyme species. So simple inhibition of NTE activity is not the critical reaction, but combined with aging reaction it may be an integral part of delayed neuropathy (Aldridge and Reiner, 1972) & (Cohen and Oosterbaan, 1963).

The relationship between delayed neurotoxicity and lipid metabolism has been extensively studied by quantitative and qualitative analysis of lipid content in affected and normal nerves and by comparison of phospholipids in normal and demyleinated tissue (Austin, 1957) & (Joel et al., 1967) & (Majno and Karnovsky, 1961) & (Morazain and Rosenberg, 1970) & (Nelson and Barnum,1960) & (Porcellati, 1971) and it was found that there was no relationship between lipid or phospholipid metabolism and delayed neurotoxicity.

Inhibition of a protein capable of degrading a lytic component would prolong the existance of a cell damaging agent e.g. Lecithin is degraded by phospholipase A_1 to the lytic molecule lysolecithin.

The latter is rapidly degraded through two mechanisms:

1. Phospholipase A_2 which yields glycerylphosphoryl choline.
2. An acylating enzyme which yields lecithin.

Inhibition of the former in absence of compensating activity of the latter should result in the prolongation of the presence of lysolecithin. The key to this proposed mechanism is the relationship of NTE and phospholipase A_2 (Baron, 1981).

It has been reported that phospholipase A_2 is present in a wide variety of animal tissues, many of which also contain NTE (Thompson, 1965). If the NTE and phospholipase A_2 are one and the same, a basic biochemical lesion might be defined. The acylation reaction of lysolecithin to lecithin may be the protective mechanism that delay the onset of axonopathy but does not completely prevent it.

Once such a lytic reaction occurred focally, the release of proteins such as B-glucoronidase and B-galactosidase as well as other phospholipids would readily

serve to rapidly degrade the axon and ultimately the myelin sheath.

In a study made for comparison of inhibitory activity of various organophosphorus compounds against cholinesterase and neurotoxic esterase of hens with respect to delayed neurotoxicity, a variety of organophosphorus compounds with or without delayed neurotoxicity were examined for inhibitory power against NTE and AChE. of hen brain *in vitro* and *in vivo*.

Single oral administration of a delayed neurotoxic EPN, leptophos and Triortho-cresyl-phosphate (TOCP) caused more than 80% inhibition of NTE at neurotoxic doses, but non delayed neurotoxic methyl parathion, fenitrothion and cyanophos caused weak inhibition at near lethal doses which give rise to severe inhibition of brain AChE.

A delayed neurotoxic dose of (-) -EPN caused more severe inhibition of brain NTE compared to the same dose of non delayed neurotoxic (+) - isomer.

A few compounds produced severe inhibition of NTE at non delayed neurotoxic doses. Hens paralysed by repeated administration of a low level of leptophos showed significant decreases in NTE activity of brain and spinal cord. (Ohkawa et al., 1980).