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# ALKYL PYRIDINIUM SALT, 2-CARBOXALDEHYDE OXIMES AND PROCESS OF PREPARATION

Irwin B. Wilson, Fair Lawn, N. J., and Sara Ginsburg, and David Nachmansohn, New York, N. Y., assignors to the United States of America as represented by the Secretary of the Army

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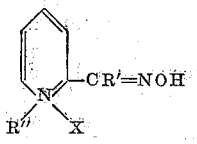
The present invention provides new compositions of matter which have been found to have antidotal properties for the irreversible inhibitors of cholinesterase.

The chemical lesion in alkylphosphate intoxication is the inhibition of acetylcholinesterase, although in some cases there may also be a reaction with the acetylcholine receptor. Once this causal connection had been established it became important to understand the exact reaction mechanism between acetylcholinesterase and alkylphosphates. Studies based on the molecular forces acting between acetylcholine and the enzyme led to an understanding of the hydrolytic process. This analysis made it possible to elucidate the exact mechanism of the reaction between alkylphosphates and enzyme.

According to these studies, in the physiological action of acetylcholinesterase an acetyl enzyme is formed which then reacts with $H_2O$ to form acetate and enzyme. In the reactions with alkylphosphate a phosphorylated enzyme is formed which, however, does not react with $H_2O$; the phosphoryl group remains attached to the enzyme and the enzyme is inhibited. Since it is a vital enzyme for nerve function, death ensues.

The antidote has been developed on the premise that nucleophilic agents should be able to dephosphorylate the enzyme and thereby repair the chemical lesion. Hydroxylamine was found to reactivate the enzyme in vitro. However, the action is slow and the required concentration is high. Acetylcholinesterase shows a specificity toward quaternary nitrogen derivatives and it was reasoned, therefore, that the activity of a hydroxylamine derivative could be promoted by the incorporation in the molecule of a quaternary ammonium structure at a suitable atomic distance.

It has been established in practice that a new class of oximes containing a positively charged nitrogen atom of the general formula below have proved to be very effective as a specific antidote against lethal alkylphosphate intoxication:




where R' represents H or alkyl and R'' represents methyl or ethyl groups. These compounds are salts where the anionic part ($X^-$) is not critical and can be a halogenide, an organic sulfate, and so on. It has not yet been established whether this new class has the syn- or the anti-configuration.

Preparation of these compounds can be achieved using two general methods:

(*a*) By action of alkyl halides, sulfates, toluene sulfonates or other N-methylating agents on tertiary pyridine oximes, or,

(*b*) By action of hydroxylamine on quaternary aldehydes and ketones.

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Tertiary pyridine oximes can be prepared in the general way, either by reacting aldehydes or ketones with hydroxylamine at slight acid or alkaline pH or by action of nitrous acid or its esters on pyridine compounds containing active methylene groups, or by reaction between a pyridine nucleus and mercuric fulminate or by interaction of diazonium salts with formaldoxime or acetaldoxime. The subsequent alkylation can be done either at room or elevated temperatures with or without solvents.

The quaternary aldehydes are prepared by applying methylating agents to aldehydes or ketones or by preparing quaternary derivatives containing active methylene groups and reacting these with nitrous acid derivatives, e. g. p-nitroso-dimethyl aniline and subsequent saponification.

Reaction of these aldehydes or ketones with hydroxylamine is effected in aqueous or alcoholic solutions at temperatures between 0° and 100° C. and neutral or slightly acidic pH.

Prior to the present invention, the class of oximes containing a positively charged nitrogen atom of the general formula, as previously shown, was not known so that the novel process of the present invention includes the synthesis of these compounds.

The procedures are further illustrated by the following examples without being restricted thereto:

## EXAMPLE I (2-PYRIDINE ALDOXIME METHIODIDE)

2-pyridine aldoxime 3.05 g., nitrobenzene 10 ml., and methyliodide 20 ml. were refluxed for 3 hours and left at room temperature overnight. This is mixed with 40 ml. acetone and filtered and rinsed with acetone. The yield is 88 percent of theory. Recrystallization from methanol produced bright yellow needles having a melting point of 224–225° C. It is very soluble in water, fairly soluble in hot alcohols, poorly soluble in cold alcohols and insoluble in ether and acetone.

## EXAMPLE II (4-PYRIDINE ALDOXIME METHIODIDE)

4-pyridine aldoxime, 1.22 g., was dissolved in acetone 20 ml., and methyliodide, 20 ml., was added. The quaternary salt started to precipitate very shortly. After several hours it was filtered off and washed with acetone. The yield was 99 percent of theory, forming bright yellow crystals having a melting point of 181–183° C.

## EXAMPLE III (METHYL-2-PYRIDYL KETOXIME METHIODIDE)

The quaternary ketone was prepared from methyl-2-pyridyl ketone with excess of methyliodide in acetone at room temperature producing faint yellow crystals having a melting point of 163–164° C. This ketone, 6.6 g., was reacted with aqueous solution of $NH_2OHHCl$, 2.6 g., neutralized with NaOH to pH 6–7. After testing for 15 minutes on steam bath the water was evaporated under reduced pressure and the almost dry residue extracted with hot absolute ethanol to eliminate mineral salts. On cooling the oxime crystallized. The overall yield was 45 percent, producing yellow needles having a melting point of 213–214°.

Certain phosphate esters such as tetraalkylpyrophosphates dialkyl-p-nitrophenyl phosphates, and dialkyl fluorophosphates, are potent irreversible inhibitors of acetylcholinesterase and esterases in general. The reactivation of alkylphosphate-inhibited acetylcholinesterase is of both practical and theoretical importance. It is of practical interest because the most potent chemical warfare gases and some powerful insecticides are alkylphosphates and their lethal action is due to the inhibition of acetylcholinesterase. It is of theoretical interest because the mech-