MEYER AND MISCH[1]	a = 8.35 Å,	b = 10.3 Å,	c = 7.9 Å,	β = 84
LEGRAND[9]	8.16	10.28	7.83	84 20′
KIESSIG[11]	8.17	10.31	7.84	84 5′

The proposed change cannot appreciably alter the intensity of the (002) spot on the X-ray diffraction diagram so that the qualitative evaluation of the intensities of the X-ray interferences by MEYER AND MISCH[1] does not exclude such a possibility. It yields a crystal lattice of cellulose I which is in better accord with the morphology of the microfibrils observed in the electron microscope than the classical model. The lattice of K. H. MEYER would demand a pronounced lamination of the native cellulose parallel to the plane (002), and the cohesion of the lattice perpendicular to it would be so weak that (002) ought to be a plane of cleavage. Further, (002) would not only be a plane of lamination, but also a plane of preferred growth so that a foliar lattice would result. None of these expectations proves to be correct. On the contrary, the diagonal plane (101) is the plane of growth, lamination and cleavage. Whilst in the old model all hydrogen bonds were concentrated in one plane demanding a pronounced sheet-like habit, in the new crystal lattice these bonds work in such a way that the chains are tied together in the two planes (101) and (10$\bar{1}$), producing a fibrillar and not a foliar habit. However, the strength of the bonds in the (101) plane is appreciably stronger than in the (10$\bar{1}$) plane, according to the distance of the oxygen atoms yielding hydrogen bonds, which is 11 % less in the (101) than in the (10$\bar{1}$) plane. As a result there is a certain tendency to form ribbon-shaped fibrils.

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Received June 15th, 1955

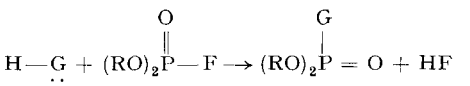
A powerful reactivator of alkylphosphate-inhibited acetylcholinesterase*

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 mechanism of inhibition and of reactivation is very closely related to the mechanism of enzymic hydrolysis[1]. This enzyme contains two sites, (i) an anionic site which contributes to the catalytic activity by binding and orienting molecules containing substituted ammonium structures, and (ii) an esteratic site which interacts with the ester function and is primarily responsible for the hydrolytic activity. During the hydrolysis of a carboxylic ester a basic group in the esteratic site is acylated to form an acyl-enzyme as intermediate. Acetyl-enzyme (from acetate-esters or anhydrides)

* This work was supported in part by the Medical Research and Development Board, Department of the Army, Office of the Surgeon General, Contract No. DA-49-007-MD-37, and in part by the Division of Research Grants and Fellowships of the National Institutes of Health, Grant B-573, United States Public Health Service.

rapidly reacts with water to produce acetic acid and to regenerate the free and active enzyme. The alkyl phosphate inhibitors react with the same basic group to form a dialkyl phosphoryl enzyme which however reacts only very slowly with water.

The inhibitory reaction is illustrated for a fluorophosphate:



active enzyme inhibitor inhibited enzyme

where H–G represents the esteratic site containing an acidic group (H) and a basic group (..). Theory predicts that nucleophilic reagents should dephosphorylate the enzyme and thus restore its activity. When R = ethyl (inhibitor = diethyl fluorophosphate or tetraethyl pyrophosphate (TEPP)) reactivation is readily accomplished by a large number of suitable compounds. When R = *iso*propyl (inhibitor = di*iso*propyl fluorophosphate (DFP)) reactivation is more difficult.

Experiments show that the anionic site survives the inhibition of the enzyme and can contribute to the reactivation process. It is therefore to be expected that a very good reactivator might be produced by combining in the same molecule an intrinsically good functional group and a suitably located quaternary ammonium structure. Experiments with a number of reactivators including hydroxamic acids have shown that the reactivating activity can indeed be promoted by interaction of a cationic center with the anionic site[2].

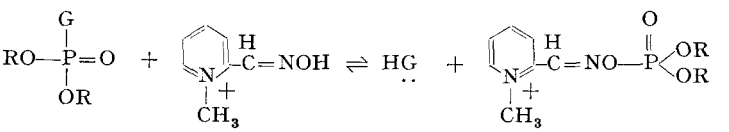
These studies also showed that the intrinsic activity could be augmented by a pyridine nucleus particularly if the functional group were in the 2-position[2]. Therefore, studies were carried out with other hydroxylamine derivatives, in particular oximes, since it was known that simple oximes are active. To test the effect of a pyridine nucleus, pyridoxal oxime was studied and found to be quite active*. We therefore prepared

(I) (II)

2-pyridine aldoxime (I) and 2-pyridine aldoxime methiodide (II)

in order to evaluate the effect of introducing a quaternary structure. Compound II was found to be extremely active in reactivating the inhibited enzyme formed with two representative inhibitors, tetraethylpyrophosphate (TEPP) and di*iso*propyl fluorophosphate (DFP). Compound I had only a low activity.

The results are presented in Table IA. From these results it appeared that measurements with TEPP might not be rate measurements but rather the extent of reactivation achieved at equilibrium of the reaction:



inhibited enzyme reactivator active enzyme postulated phosphorylated oxime

To test this possibility the inhibited enzyme was diluted 400 times (instead of 7.5 times) before the reactivator was added. Under these circumstances the postulated phosphorylated oxime should be greatly reduced and the equilibrium displaced to the right. The results so obtained, Table IB, are consistent with the assumption of equilibrium. High reactivations are obtained with very low reactivator concentrations. With TEPP even at these low concentrations it appears that the rate is much less than 1 minute and that the measurements constitute equilibrium values. The DFP data seem to indicate a rate, but the situation is not clear.

The quaternary oxime is a million times better than the non-methylated compound and 50,000 times better than picolinohydroxamic acid in reactivating TEPP inhibited enzyme. The reactivation appears to be approaching enzyme speeds. It has not yet been established, whether our compound has the syn- or the anti-configuration.

* Kindly supplied by Dr. Karl Pfister, Merck & Co., Inc.

TABLE I

PERCENT REACTIVATION OF ALKYLPHOSPHATE-INHIBITED ACETYLCHOLINESTERASE WITH 2-PYRIDINE ALDOXIME METHIODIDE

The reactivations were carried out as follows: 0.2 ml of enzyme solution prepared from *Electrophorus electricus* was treated with 0.01 ml of TEPP or DFP solution (20 γ/ml), and diluted after 1 hour in the cold to 1.5 ml. This solution was used as stock for reactivation; to 0.2 ml were added 0.2 ml of reactivator solution of suitable concentration in 0.015 *M* phosphate buffer (pH 7) and 0.007 *M* EDTA. After suitable incubations (1', 5', 11') the reactivated solution was diluted to 50 ml with water and 1 ml was added to the manometric vessels for assay. The total enzyme dilution was 5625 fold. In part B the inhibited enzyme was diluted to 80.0 ml instead of 1.5 ml.

A. Diluted 7.5 times

	TEPP		*DFP*	
	1'	5'	1'	5'
$5 \cdot 10^{-3}$ *M*	—	—	55	82
$2 \cdot 10^{-3}$	—	—	43	66
10^{-3}	94	94	—	—
10^{-4}	41	48	—	—
10^{-5}	8	9.5	—	—

B. Diluted 400 times

	1'	5'	1'	11'
10^{-4}	—	—	38	53
10^{-5}	85	89	9	12
10^{-6}	25	29	—	—

Most reactivators react directly with TEPP and DFP[4]. This is also the case with the quaternary oxime. The rate as judged by acid production was fairly rapid but not extraordinary; with 0.01 *M* oxime and 0.002 *M* phosphate anhydride the time for 50% reaction at 25° C and pH 7.4 was 12 and 20 minutes for TEPP and DFP respectively.

The nicotino- and picolinohydroxamic acids proved already to be of value as antidote of the so-called "nerve gas" in animals. The quaternary oxime is thousands of times more potent as a reactivator; therefore, the possibility of its use as a powerful antidote appears promising.

The authors wish to acknowledge the assistance of Miss CAROLE QUAN and to express their thanks to Dr. DAVID NACHMANSOHN for his continuous interest.

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Received July 13th, 1955