THE ENZYMIC HYDROLYSIS OF ORGANOPHOSPHORUS COMPOUNDS

by

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During studies on the mechanism of cholinesterase inhibition by organophosphorus compounds we have demonstrated that several of the most active of these enzyme inhibitors are enzymically hydrolysed. According to previous studies, enzymes exist that catalyse the hydrolysis of di*iso*propylfluorophosphate (DFP)[1,2,3] and dimethyl-*p*-nitrophenyl phosphate[4]. These observations have been confirmed in our laboratory and extended to several organophosphorus compounds, especially those containing a P–N bond; in the first place the dimethylamido-ethoxy-phosphoryl cyanide or tabun (for literature, see ref.[5]) has been used as substrate in these enzyme studies. The results obtained are briefly reported.

There exists in the plasma of various animal species (rabbit, dog, horse, man, cat, rat, cow, and guinea pig) an enzyme, tentatively called "phosphatase", which hydrolyses tabun. The rabbit plasma is particularly active. Using human blood serum, the "phosphatase" has been separated from cholinesterase and partly purified.

Among tissues (rabbit, pig, cow), kidney, adrenal gland, and liver cause appreciable enzymic hydrolysis of tabun. Especially active is the adrenal gland of rabbit, the cortex being more active than the medulla. "Phosphatase" activity has also been observed for lung, brain, and small intestine. Preparations known to contain acetylcholinesterase as the only esterase (*e.g.*, electric tissue, cobra venom) do not hydrolyse tabun or other organophosphorus compounds. Human erythrocytes have a low "phosphatase" activity.

The "phosphatase" of human serum is not identical with the DFP hydrolysing enzyme from kidney or adrenal gland. There is, for instance, no activation by Mn^{++} of the serum enzyme as compared with the "phosphatase" of kidney (cf. ref.[3]).

Systems containing cholinesterase and "phosphatase" as enzyme mixture, and acetylcholine and an organophosphorus compound as substrate-inhibitor mixture have been studied in detail. It was found, among other things, that the presence of "phosphatase", *e.g.*, in human serum, has a definite influence on the inhibition (inactivation) of cholinesterase by organophosphorus compounds. Thus the pI_{50} value of tabun for the inhibition of human serum cholinesterase is 7.89 for the original serum, and 8.31 when "phosphatase" is absent. Physostigmine and prostigmine (10^{-3} *M*) do not affect the "phosphatase" activity.

During the hydrolysis of tabun by the action of "phospbatase", hydrocyanic acid is liberated. It is known that this reaction takes place spontaneously in alkaline solution[6]. The amount of cyanide parallels the amount of carbon dioxide evolved from a bicarbonate system both for enzymic and spontaneous hydrolysis. The CO_2 liberation is due to the strongly acid reaction product, dimethylamido-ethoxy-hydroxy-phosphine oxide. One mole of this acid (determined by the CO_2 production) is formed per mole tabun hydrolysed. There is no release of dimethylamine, ethanol, or inorganic phosphate.

Some general properties of the "phosphatase" have been determined, such as its behaviour in dialysis, pH stability (optimum stability at pH 6.5–7.5), and the MICHAELIS constant for the hydrolysis of tabun by purified ($pK_S = 2.67$) and crude enzyme ($pK_S = 2.46$) of human serum.

Specificity studies have shown that the chloride analogue of tabun is hydrolysed more rapidly than the cyanide analogue, also that the methoxy compound is hydrolysed at the highest rate compared with its higher homologues. Other more generally known organophosphorus compounds, such as DFP, TEPP, mintacol, etc., have been included in these studies.

Acetylcholinesterase completely or almost completely inhibited by tabun was reactivated by incubation with "phosphatase". This reaction is supposed to be similar to the reaction mentioned above in which cyanide is split off from tabun. The phosphorylation of cholinesterase, the reaction which is responsible for the inactivation of that enzyme[7], most certainly takes place at a hydroxy group (of serine, or one of the phenolic groups of a dihydroxyphenyl derivative; cf. ref.[8]). The "phosphatase" cannot split a P–N bond, and therefore this type of chemical bond is most unlikely to exist between the phosphorus containing part and the esteratic site of the inactivated cholinesterase complex.

A full account of these investigations will be published later as a series of papers in *Acta Chem. Scand.*

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IDENTIFICATION OF TWO METABOLITIES OF ISONIAZID (ISONICOTINOYLGLYCINE AND 1-ISONICOTINOYL-2-ACETYLHYDRAZINE) BY PAPER CHROMATOGRAPHY IN RAT URINE

by

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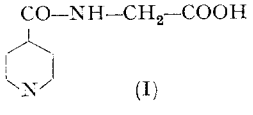
During the comparative study of the metabolism of isonicotinic acid hydrazide (isoniazid) and isonicotinoyl-hydrazino-methanesulfonic acid (Neotizide), the urine of rats, which had been injected with these two drugs, was examined by paper chromatography. It was found that another compound (Compound X) was excreted, besides isoniazid and isonicotinic acid[1].

This substance was detected on the chromatogram not only by the methods already described, that is, by absorption of short wave-length U.V. light (Mineralight Lamp), and yellow colour after treatment with cyanogen bromide and ammonia[1], but also by strong blue violet colour after reaction with benzidine and cyanogen bromide[2], and by the characteristic green colour it gives when treated with picryl chloride and ammonia[3].

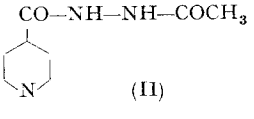
It was seen at once that Compound X was not isonicotinamide, as we supposed at first, because the chromatographic band of the latter has clearly different R_F values with the solvents tried.

It was then thought by analogy with that which occurs in the metabolism of nicotinic acid (which is partly eliminated in the urine combined with glycine as nicotinuric acid), that compound X might be isonicotinoylglycine (I). This hypothesis was confirmed by further research. In fact, when comparing the R_F values observed by paper chromatography in different solvents, compound X was found to behave as isonicotinoylglycine. This substance was prepared by the method of ROHRLICH[4] for nicotinoylglycine. Also the colours resulting from treatment with different reagents appeared to correspond, especially the green colour with picryl chloride and ammonia.

Further confirmation was given when, after elution of the chromatographic band detected by absorption of U.V. light, the eluate was hydrolysed with 6 *N* hydrochloric acid for four hours in sealed tube at 120°. Two paper chromatograms of the hydrolysate were run: one using as a solvent *n*-butanol, saturated with 0.5 *N* aqueous acetic acid, and using as detecting reagents benzidine and cyanogen bromide; and another using phenol: water (80:20) as a solvent, and ninhydrin as detecting reagent. In the former chromatogram isonicotinic acid was found; in the latter chromatogram glycine.



(I)



(II)

Another product of the metabolism of isoniazid was detected in the course of this research. This is a substance which was not noticed in the early experiments[1] because with the solvent used (*iso*amyl alcohol saturated with 0.5 *N* acetic acid) its chromatographic band overlaps that of isonicotinic acid. On the other hand, using a mixture of *iso*propanol and water (85:15) the compound was clearly separated from the other metabolites of the isoniazid, and was identified as 1-isonicotinoyl