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The Hydrolysis of Dimethylamido-ethoxy-phosphoryl Cyanide (Tabun)

LENNART LARSSON

Division of Organic Chemistry, Research Institute of National Defence, Dept. 1, Sundbyberg 4, and Institute of Organic Chemistry and Biochemistry, University of Stockholm, Sweden

The hydrolysis of the cyanide group in the biochemically very active compound dimethylamido-ethoxy-phosphoryl cyanide, generally known as Tabun, has previously been investigated by Holmstedt [1]. The hydrolysis of some other phosphorus compounds, in particular diisopropoxy-phosphoryl fluoride (DFP) [2,3], tetraethylpyrophosphate (TEPP) [4,5], and the dimethylamides of phosphoric acids [6], has been studied closely. In the latter compounds it has been found that the P—N linkage is easily broken by acids, but is extremely stable to alkalis. By means of a qualitative infra-red spectrophotometric method the present author recently established that in the hydrolysis of Tabun both the dimethylamino group and the cyanide group are split off, but that splitting off of the ethoxy group seems to be negligible [7].

The purpose of the present investigation was to determine the reaction mechanism of the hydrolysis of Tabun, and the variation with pH of the rates of removal of the dimethylamino and cyanide groups in acidic solutions. By quantitatively determining the amount of dimethylamine and hydrocyanic acid formed during the hydrolysis and simultaneously following the decrease in toxicity it has been possible to establish that the hydrolytic decomposition of Tabun proceeds in two parallel two-stage reactions.

Most of the organic phosphorus derivatives are powerful inhibitors of the enzyme cholinesterase, and several investigations have been carried out to correlate the inactivation of cholinesterase *in vitro* with the toxicity *in vivo* [1]. Such a relationship is usually present, but some exceptions to this rule are found. In this connection the decrease in toxicity has been compared with the cholinesterase inhibition determined concurrently during the hydrolysis of Tabun in aqueous solution.

EXPERIMENTAL

The hydrolysis of Tabun, synthesized in this institute [1], was measured in McIlvaine's standard buffer solutions at pH 3.0, 4.0, and 5.0 and in one case in aqueous solution. The pH of the solution was measured at the beginning and the end of the hydrolysis, and found to remain constant. 0.200 ml of Tabun, measured out with an AGLA micrometer syringe, was diluted to 100.0 ml with buffer solution or water. The concentration of Tabun was thus $13.29 \cdot 10^{-3}$ *M*. The reaction mixture was kept in a water bath at 25.0 ± 0.2° C.

Determination of dimethylamine. The dimethylamine liberated was determined by the colorimetric method described by Dowden [8]. The principle of this method is to react the dimethylamine with carbon disulfide thereby converting it into dimethyl-dithiocarbamic acid, the cupric salt of which has an amber tint and is rather soluble in benzene. The procedure is described in detail in a paper quoted above [7].

Determination of hydrocyanic acid. The hydrocyanic acid formed during the hydrolysis was determined iodometrically as described by Cupples [9]. At appropriate times 4.00 ml aliquots were diluted with 50 ml of 0.2 *M* sodium hydrogen carbonate solution and titrated with 0.01 *M* iodine solution using starch as indicator. This method, however, is not quite quantitative, so it was standardized against solutions containing known amounts of cyanide. In addition the results were corrected for the small amount of hydrocyanic acid which evaporated from the reaction mixture. This correction was performed at intervals by totally hydrolyzing an aliquot of the sample with sodium hydroxide and then titrating with iodine in the manner above.

Toxicity test. The experimental method adopted to follow the hydrolytic breakdown of the Tabun itself was to determine at appropriate intervals the toxicity of the reaction mixture. This was done by intraperitoneal injection in white mice weighing between 20 and 32 grams. Median lethal doses (LD_{50}) were calculated by the method of Finney [10], using mortality data obtained from groups of ten mice at each of five dose levels. The mortality was estimated 30 minutes after the moment of injection. The LD_{50} value at time zero was obtained by extrapolation. From the LD_{50} values obtained the amount of hydrolyzed Tabun, expressed in per cent, was calculated from the expression:

$$\frac{LD_{50_t} - LD_{50_0}}{LD_{50_t}} \cdot 100$$

Determination of anticholinesterase activity. The anticholinesterase activity of the reaction mixture was determined by the electrometric method described by Tammelin and Strindberg [11]. The principle of this method is to determine the acetic acid produced by the splitting action of cholinesterase on the substrate acetylcholine in terms of the change in pH in the presence of a standard buffer solution. Cobra venom was chosen as the source of cholinesterase, as it gives the most reproducible values and has a high enzymatic activity [12]. The molar concentration of the inhibitor during the pH-determination which caused 50 per cent inhibition of the enzymatic activity (I_{50}) was used as the measure of the anticholinesterase activity. The I_{50} values were calculated according to Augustinsson [13] by plotting the ratio v/v' against the inhibitor concentration, where v and v', expressed by $d(pH)/dt$, represent the observed velocities of the hydrolysis of acetylcholine in the absence and presence of inhibitor respectively.

Samples were withdrawn from the hydrolysis solution and diluted to a suitable concentration for the pH-determination. The I_{50} values were then calculated on the initial

Tabun concentration. The hydrolysis of Tabun in per cent was calculated from the expression:

$$\frac{I_{50_t} - I_{50_0}}{I_{50_t}} \cdot 100$$

The reaction mixture in which the pH-determinations were made, had the following composition: 3.00 ml of Michel's buffer solution [14] + enzyme (according to Augustinsson [12]: 0.1 mg of cobra venom per 2.00 ml of reaction mixture); 2.34 ml of redistilled water; 0.66 ml of redistilled water or of inhibitor, which was added 30 minutes before the acetylcholine solution; and 0.60 ml of acetylcholine solution (0.110 g of acetylcholine iodide per 5.00 ml of water).

RESULTS AND DISCUSSION

Figures 1 and 2 illustrate the liberation of dimethylamine and hydrocyanic acid respectively at pH 3.0, 4.0, and 5.0. From the curves it is obvious that the fission of the P—N linkage is catalyzed by hydroxonium ions, in agreement with the results obtained by Heath and Casapieri [6]. On the other hand the velocity of hydrolysis of the cyanide group increases with increasing pH as demonstrated by Holmstedt [1].

The reaction mechanism of the hydrolysis was studied at pH 5.0. Because both dimethylamine and hydrocyanic acid are liberated, there are three alternative courses for the hydrolysis:

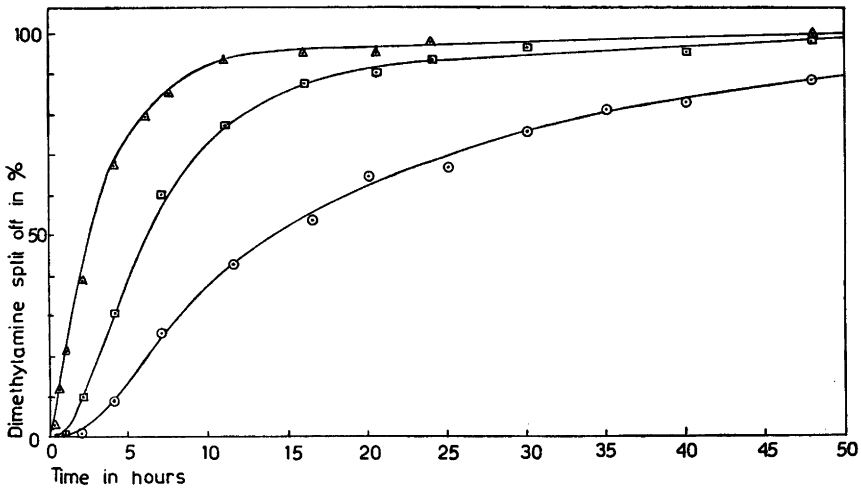
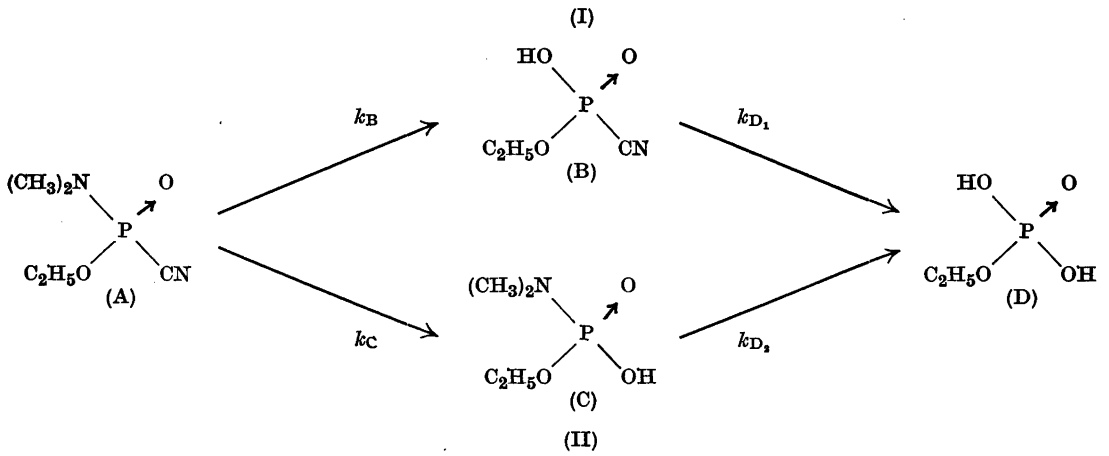


Fig. 1. The rate of formation of dimethylamine during the hydrolysis of Tabun. △ *at pH 3.0,* ⊡ *at pH 4.0,* ⊙ *at pH 5.0.*



Either it can proceed as a single two-step reaction (I) in which the dimethylamino group is first split off forming B which is then broken down to D and hydrocyanic acid, or inversely (reaction II), or finally by a combination of both these routes. To establish in which of these possible ways the hydrolysis proceeds, the decreasing toxicity of the reaction mixture was studied. The toxicity of Tabun is much higher than that of the hydrolysis products and therefore the toxicity of the reaction mixture depends essentially only on the

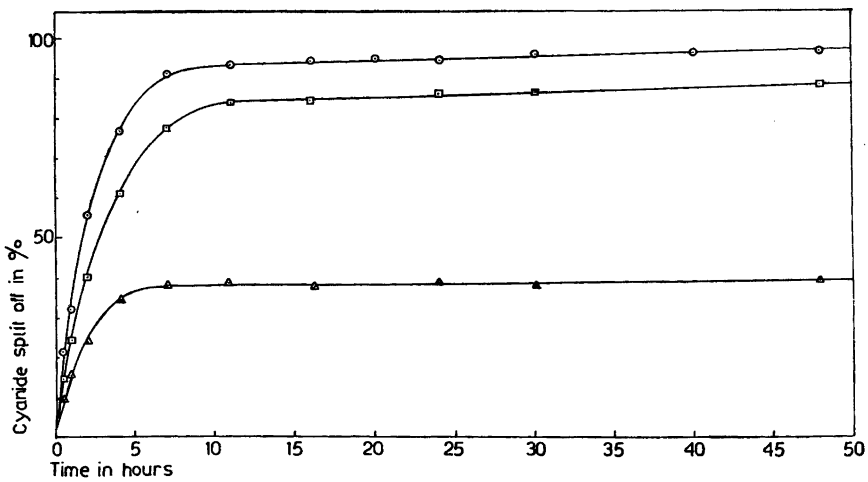


Fig. 2. The rate of formation of hydrogen cyanide during the hydrolysis of Tabun. △ *at pH 3.0,* ⊡ *at pH 4.0,* ⊙ *at pH 5.0.*

Tabun concentration. Near the end of the reaction when the Tabun concentration is very low, the hydrolysis products will however influence the toxicity. If the hydrolysis proceeds by either route (I) or (II), the dimethylamine or hydrocyanic acid respectively should be liberated at the same rate as the toxicity decreases. However, this is not the case, and consequently the hydrolysis must proceed by both of the two parallel routes in the scheme above.

At constant pH all the individual reactions would be expected to proceed as first order reactions. If log c_A (c_A calculated from toxicity tests) is plotted against time (t), a straight line is obtained as expected. From the experimentally determined values of dimethylamine and hydrocyanic acid the concentrations c_B, c_C, and c_D can be calculated:

$$c_{(CH_3)_2NH} = c_B + c_{D_1} + c_{D_2} \tag{1}$$

$$c_{HCN} = c_C + c_{D_1} + c_{D_2} \tag{2}$$

$$c_{A_0} - c_A = \Delta c_A = c_B + c_C + c_{D_1} + c_{D_2} \tag{3}$$

From these equations we obtain:

$$c_B = \Delta c_A - c_{HCN} \tag{4}$$

$$c_C = \Delta c_A - c_{(CH_3)_2NH} \tag{5}$$

$$c_D = c_{D_1} + c_{D_2} = c_{HCN} + c_{(CH_3)_2NH} - \Delta c_A \tag{6}$$

The amount of A measured as LD_{50} and the amounts of B, C, and D as calculated from these equations are recorded in Fig. 3.

For the different reaction steps the following differential equations can be set up:

$$dc_A/dt = -k_B c_A - k_C c_A = -(k_B + k_C)c_A = -k_A c_A \tag{7}$$

$$dc_B/dt = k_B c_A - k_{D_1} c_B \quad (8a) \quad \text{and when } c_A = 0 \quad dc_B/dt = -k_{D_1} c_B \tag{8b}$$

$$dc_C/dt = k_C c_A - k_{D_2} c_C \quad (9a) \quad \text{and when } c_A = 0 \quad dc_C/dt = -k_{D_2} c_C \tag{9b}$$

Integration gives:

$$c_A = c_{A_0}\, e^{-k_A t} = c_{A_0}\, e^{-(k_B + k_C)t} \tag{10}$$

$$c_B = \frac{k_B c_{A_0}}{k_{D_1} - k_A} \left(e^{-k_A t} - e^{-k_{D_1} t}\right) \tag{11}$$

$$c_C = \frac{k_C c_{A_0}}{k_{D_2} - k_A} \left(e^{-k_A t} - e^{-k_{D_2} t}\right) \tag{12}$$

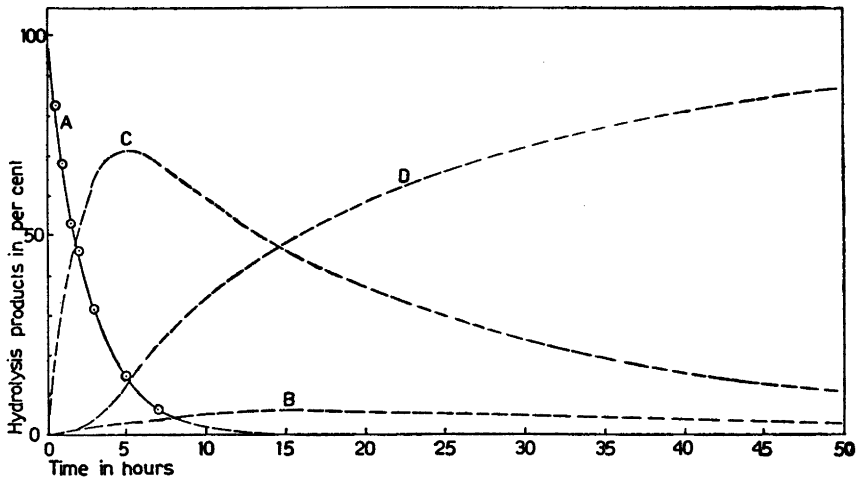
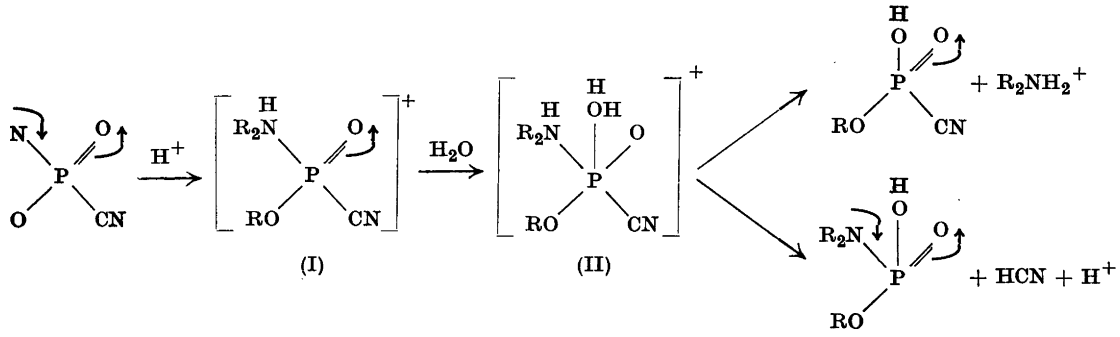


Fig. 3. The hydrolysis of Tabun at pH 5.0. A is measured as LD_{50} *and B, C, and D are calculated from the equations 4—6.*

If log c_A is plotted against t, a straight line is obtained as mentioned above, and from the slope of this line the velocity constant of the decomposition of Tabun was calculated by the method of least squares. The numerical value of k_A obtained was 0.390 hours^{-1}. The velocity constants k_{D_1} and k_{D_2} were calculated in the same way by plotting log c_B and log c_C respectively against t beginning after 16 hours, when all the Tabun is assumed to be hydrolyzed. In agreement with equations (8b) and (9b) straight lines were obtained. The values of k_{D_1} and k_{D_2} so obtained were 0.021 hours^{-1} and 0.043 hours^{-1} respectively. By inserting the experimental values for c_B into equation (11) values for k_B were calculated. The calculation of k_C was performed in the same way from equation (12) and the following numerical average values were obtained: $k_B = 0.01$ hours^{-1} and $k_C = 0.38$ hours^{-1}. The accuracy of the last two values will be rather low because of the nature of the calculations.

The interpretation of the mechanism of hydrolysis of phosphorus halides and organic esters of phosphoric acid is that a molecule of water attacks the phosphorus atom, one of the vacant 3d orbitals of the phosphorus being utilized for this purpose [3,15]. Depending upon the acidity of the solution, the intermediate compound thus formed either accepts or loses a proton forming an unstable ion which decomposes spontaneously to stable products. This mechanism, however, is not applicable to phosphoramides, because the amino group exerts a large electron-donating electromeric effect which will interfere with the coordination of the molecule of water to the phosphorus atom [6,16].

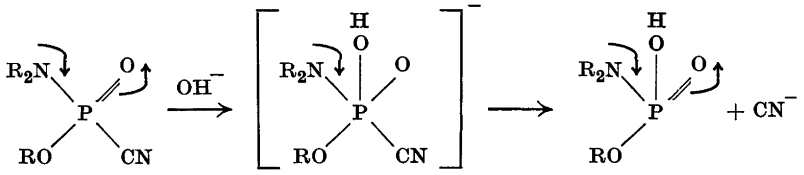
For this reason Day and Ingold's [17] kinetic scheme for the hydrolysis of carbonic esters seems to be more applicable to the hydrolysis of Tabun, and in acid solution the following mechanism is conceivable:



(I) (II)

In the first step which is a fast reaction, a proton is added to the nitrogen atom of the amino group (I). The positive electromeric effect is thus nullified and the hydration of the phosphorus atom is facilitated. The hydrated intermediate (II), which is formed slowly, decomposes spontaneously. In this case either the dimethylamino group or the cyanide group can be split off.

The base-catalyzed hydrolysis probably takes place by the following mechanism:



(III)

The rate-controlling step is a nucleophilic attack on the phosphorus atom by the hydroxyl ion to form the intermediate ion (III). This step is followed by the elimination of the cyanide ion, while the dimethylamino group is not removed in this reaction.

Finally the hydrolysis of Tabun has been studied with regard to decreasing toxicity and anti-cholinesterase activity. From Table 1 it appears that the decrease in toxicity during the hydrolysis agrees well with the decrease in cholinesterase inhibition.

Table 1. The hydrolysis of Tabun determined as LD_{50} and I_{50}.

Time in hours	LD_{50} μM/kg	I_{50} M/l	Hydrolyzed Tabun in % determined as	
			LD_{50}	I_{50}
0	*3.72	*$2.93\ 10^{-9}$	0	0
0.5	4.44	$3.42\ 10^{-9}$	16.2	16.7
1.5	5.95	$4.33\ 10^{-9}$	37.5	32.4
3.0	8.63	$6.75\ 10^{-9}$	56.9	56.6
5.0	16.40	$1.06\ 10^{-8}$	77.3	72.3
6.5	25.60	$1.42\ 10^{-8}$	85.4	79.3
11.5	61.10	$5.02\ 10^{-8}$	93.8	94.5
17.0	101.63	$1.40\ 10^{-7}$	96.3	98.0

* Extrapolated value.

SUMMARY

The velocity of hydrolysis of dimethylamido-ethoxy-phosphoryl cyanide, Tabun, in regard to the dimethylamino and cyanide groups has been measured at pH 3.0, 4.0, and 5.0. It appears that the splitting off of the dimethylamino group is catalyzed by hydroxonium ions and that of the cyanide group by hydroxyl ions.

It has been shown that the hydrolysis of Tabun proceeds in two parallel two-stage reactions. The various velocity constants have been determined at pH 5.0, and the mechanism of the hydrolysis has been discussed.

Finally, it has been demonstrated that the decrease in toxicity during the hydrolysis parallels the decrease in anticholinesterase activity.

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