



Fig. 3. In experiments I and IV, trypsin is used as enzyme. In I 5 mg of fraction C-2 is added, in IV no inhibitor. In experiments II and III chymotrypsin is used as enzyme, II with addition of 5 mg of fraction C-2, III without inhibitor.

activity. It will also be seen that fraction C-2 apparently has a stronger effect than fraction C-3. No inhibitory effect could be found when mixing the other subfractions of crude seromucoid with the substrate; the deviations from the reference experiments found were within the range of the experimental error ($\pm 5\%$).

Finally the effect of fraction C-2 on hydrolysing casein with chymotrypsin was studied. Fig. 3 illustrates these experiments including a control experiment with trypsin. From the figure it will be seen that fraction C-2 showed no inhibitory effect on chymotrypsin.

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The Alkaline Hydrolysis of Two Sarin Analogues and of Tabun

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In an earlier report the alkaline hydrolysis of *isopropoxy-methyl-phosphoryl fluoride* (Sarin) has been studied, and the influence of substituents in the alkoxy and alkyl groups have been discussed¹. In this paper the rate of the alkaline hydrolysis of two analogous compounds, *dimethylamido-methyl-phosphoryl fluoride* and *ethylthio-methyl-phosphoryl fluoride*, has been determined. Further it was of interest to study the alkaline hydrolysis of *dimethyl-amido-ethoxy-phosphoryl cyanide* (Tabun), the acid hydrolysis of which has earlier been examined².

The hydrolysis of the compounds was studied at various temperatures and pH-values in a 0.1 M solution of potassium chloride by means of an automatic recording titrator³ by which the pH of the solution was maintained constant during the reaction. When the hydrolysis curves obtained from the recorder were treated according to the method given by Guggenheim, straight lines were obtained indicating first-order reactions.

In the hydrolysis of the two Sarin analogues two equivalents of acid were formed. In the hydrolysis of the dimethylamino derivative the P-N bond is assumed to be quite stable at the pH-values in question⁴, and only the P-F bond can be considered to be broken. The hydrolysis products of the ethylthio derivative were analyzed by paper chromatography and only one spot indicating a phosphorus compound was obtained. When the paper was developed for sulphur one spot appeared which had the same R_F -value, 0.41, as the spot developed with the phosphorus reagent. These results indicate that only the P-F bond is split in the hydrolysis of the thio-compound also.

The first-order rate constants, k_1 , for the hydrolysis of the two Sarin analogues are given in Table 1. The second-order rate constants, k_2 , were calculated from

$$k_1 = k_2 \cdot \text{COH}^- = k_2 \cdot \frac{a_{\text{OH}^-}}{f_{\text{OH}^-}} = k_2 \cdot \frac{K_w}{f_{\text{OH}^-} \cdot a_{\text{H}^+}}$$

Table 1. The first-order rate constants for the hydrolysis of dimethylamido-methyl-phosphoryl fluoride and ethylthio-methyl-phosphoryl fluoride at various temperatures and pH-values.

Dimethylamido-methyl-phosphoryl fluoride			Ethylthio-methyl-phosphoryl fluoride		
$t^{\circ}\text{C}$	pH	$k_1 \times 10^4 \text{ sec}^{-1}$	$t^{\circ}\text{C}$	pH	$k_1 \times 10^3 \text{ sec}^{-1}$
25.0	9.50	0.422	15.0	7.00	0.813
	9.50	0.428		7.00	0.770
	10.00	1.42		7.00	0.825
	10.00	1.42		7.50	2.49
	10.50	4.14		7.50	2.32
35.0	10.50	4.29	7.50	2.53	
	9.42	1.42	25.0	7.00	2.60
	9.42	1.44	7.00	2.48	
			7.00	2.56	

The values of the activity coefficient, f_{OH^-} , and of the ionic product of water, K_w , were those reported in an earlier paper¹. The values of k_2 and the energies and entropies of activation are given in Table 3.

The low rate of hydrolysis of the dimethylamino derivative can be attributed to the mesomeric effect of the nitrogen atom. Owing to the lower electronegativity of sulphur in comparison with that of oxygen it would be expected that the rate of hydrolysis of the ethylthio derivative should be lower than that of Sarin. However, the great hydrolytic instability of the thio-compound may probably be ascribed to the ready polarisability of sulphur⁵ which also has been postulated by Heath for some related thio-compounds⁶.

It has been shown that in the acid hydrolysis of Tabun the cyano group as well as the dimethylamino group are split off². To check if the latter reaction occurred at pH 8.50 also, the reaction mixture was analyzed colorimetrically⁷ for dimethylamine when the reaction had terminated (after about 20 h). It was found that about 4% of dimethylamine had been formed. At higher pH-values the formation of dimethylamine was still less. This reaction could thus be neglected in the calculation of the rate constant for the split off of the cyano group. In the hydrolysis of Tabun less than two equivalents of alkali were consumed owing to the low dissociation constant of hydrocyanic acid. The observed rate constant, k_{obs} , was not found to be directly proportional to the hydroxyl

ion concentration as appears from Table 2. Experiments with a 0.05 M solution of potassium chloride showed that the rate of hydrolysis was independent of the concentration of this salt. From these results one could conclude that a reaction between Tabun and water molecules also takes place and the following expression was found to be valid for the hydrolysis of Tabun:

$$k_{\text{obs}} = k_{\text{H}_2\text{O}} + k_2 \cdot \text{COH}^- = k_{\text{H}_2\text{O}} + k_2 \cdot \frac{K_w}{f_{\text{OH}^-} \cdot a_{\text{H}^+}}$$

By plotting k_{obs} versus COH^- a straight line was obtained, and $k_{\text{H}_2\text{O}}$ was found to be

Table 2. The observed rate constants for the hydrolysis of dimethylamido-ethoxy-phosphoryl cyanide (Tabun) at various temperatures and pH values.

$t^{\circ}\text{C}$	pH	$k_{\text{obs}} \times 10^4 \text{ sec}^{-1}$
25.0	8.50	0.726
	8.50	0.736
	8.75	0.887
	8.75	0.921
	9.00	1.40
	9.00	1.37
	9.50	3.48
35.0	9.50	3.50
	9.00	4.01
	9.00	4.00
	9.50	11.8
	9.50	11.6

Table 3. Second-order rate constants and energies and entropies of activation of the three organic phosphorus compounds.

Compound	k_2^{15} 1 mole ⁻¹ sec ⁻¹	k_2^{25} 1 mole ⁻¹ sec ⁻¹	k_2^{35} 1 mole ⁻¹ sec ⁻¹	E_a kcal. mole ⁻¹	ΔS^\ddagger E.U.
Dimethylamido-methyl-phosphoryl fluoride	—	1.04 ± 0.04	1.99 ± 0.02	11.9 ± 0.9	-21 ± 3
Ethylthio-methyl-phosphoryl fluoride	$(1.36 \pm 0.06) \times 10^4$	$(1.95 \pm 0.05) \times 10^4$	—	6.2 ± 1.2	-22 ± 4
Dimethylamido-ethoxy-phosphoryl cyanide	—	7.49 ± 0.08	13.0 ± 0.18	10.1 ± 0.4	-23 ± 1

$(0.40 \pm 0.02) \times 10^{-4}$ sec⁻¹ at 25°C and $(0.44 \pm 0.11) \times 10^{-4}$ sec⁻¹ at 35°C. The values of k_2 and the energies and entropies of activation for the alkaline hydrolysis of Tabun are given in Table 3.

Experimental. The syntheses of the two Sarin analogues have recently been published⁸ and Tabun was synthesized according to Holmstedt⁹. In the kinetic experiments performed with the automatic titrator³ the concentrations of the dimethylamino derivative and of Tabun were 0.40 mM and of the ethylthio derivative 0.20 mM. The first one was titrated with 0.1 M sodium hydroxide and the latter two with 0.05 M sodium hydroxide. In other respects the procedure closely adhered to that described for the determination of the alkaline hydrolysis of Sarin¹. In the paper chromatography ascending technique was used and 50 μ l in portions of 5 μ l of a 1 % aqueous solution of the ethylthio derivative were placed on a filter paper (Whatman No. 1). *Solvent:* Tert-butanol: isopropanol: 2 M ammonium hydroxide (2:2:1). *Reagents:* Ammonium molybdate + hydrogen sulphide for the development of phosphorus¹⁰ and N-ethyl-maleinimide for sulphur¹¹.

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The Influence of Anharmonicity upon the Vibrational Probability Densities and Mean Amplitudes in Molecules. The General Case of a Polyatomic Molecule with Non-Degenerate Normal Vibrations

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The vibrational probability density for a general polyatomic molecule with non-degenerate normal vibrations and without accidental degeneracies has been calculated to terms of second order, making use of the Hamiltonian introduced by Darling and