

The Hydrolysis of Some Choline Esters

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Knowledge of the rate of spontaneous hydrolysis of choline esters is important both in the determination of their stability in aqueous solutions when used as drugs, and in the study of their enzymatic hydrolysis. The only choline esters which have been investigated in this respect are acetylcholine and succinylcholine (Celocurin). The hydrolysis of acetylcholine at various pH levels was studied by Hofmann¹; its dependence on the ionic concentration by Nilsson²; and, lastly, a complete investigation of its kinetics was conducted by Butterworth *et al.*³. The hydrolysis of succinylcholine at various pH levels and temperatures was studied by Tammelin⁴. The object of the present investigation was to obtain kinetic data on the hydrolysis of the iodides of acetyl- β -methylcholine (Betacholy), propionylcholine and butyrylcholine.

EXPERIMENTAL

The hydrolysis of the choline esters, synthesized by Tammelin⁴, was measured in Clark and Lub's standard buffer solutions (Kolthoff⁵) at pH 1, 2, 3, 6, 7, and 8 and at 60.0°, 75.0°, and 90.0° C. The pH levels of the solutions at those temperatures were measured with a Radiometer pH-meter, Type 24; a phosphate buffer solution and a phthalate buffer solution with known pH levels at these temperatures⁷ were used as references. The pH of the solutions was determined at the beginning and end of hydrolysis, and was found to remain constant. The ionic products of water at the various temperatures were calculated with the formula reported by Harned and Hamer⁸. The temperature variation in the thermostat was less than $\pm 0.2^\circ$ C.

Procedure. Forty milliliters of the buffer solution was brought to the thermostat temperature in a vessel fitted with a stoppered condenser. At zero time, 0.030 g of the choline ester was added and the vessel shaken, so that the compound rapidly dissolved. At intervals, 1.00 ml samples were withdrawn and analysed. The concentration of unhydrolysed choline ester was determined colorimetrically according to the method reported by Hestrin⁹. The principle of this method is to convert the choline ester with hydroxylamine into hydroxamic acid, which forms a coloured complex with ferric ions. This complex exhibits absorption maximum at 5 200 Å. The colour intensities of the samples were measured in a 10 mm cell with a Beckmann B spectrophotometer. Because Lambert-Beer's law is obeyed, the concentration of the unhydrolysed choline ester is direct proportional to the optical density.

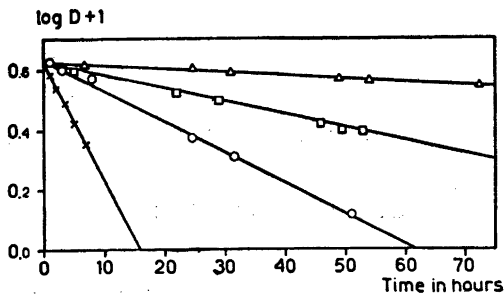


Fig. 1. Hydrolysis of acetyl- β -methylcholine at 75.0°C. \circ = pH 2.11; \triangle = pH 3.13; \square pH 5.95; \times = pH 6.91.

RESULTS AND DISCUSSION

The hydrolysis of choline esters is catalyzed by both hydronium and hydroxyl ions as ordinary, non-ionic esters. Since the reaction with water molecules is of subordinate importance compared with the reaction with hydronium and hydroxyl ions, the rate constant of the hydrolysis may be equated as

$$k = k_{\text{H}^+} [\text{H}_3\text{O}^+] + k_{\text{OH}^-} [\text{OH}^-] \quad (1)$$

In this equation k_{H^+} and k_{OH^-} are the catalytic constants for hydronium and hydroxyl ions respectively. By making use of the ionic product of water, K_w , elimination of $[\text{OH}^-]$ gives

$$k = k_{\text{H}^+} [\text{H}_3\text{O}^+] + \frac{k_{\text{OH}^-} K_w}{[\text{H}_3\text{O}^+]} \quad (2)$$

Since the hydronium ion concentration in the buffer solution remains constant during the reaction, the hydrolysis can be treated as a first order reaction, and as Lambert-Beer's law is obeyed, the rate constant at each pH was calculated from the first order equation

$$k = -\frac{1}{t} \ln \frac{D}{D_0} \quad (3)$$

where D_0 and D are the optical densities at zero time and t time, respectively. This is illustrated in Fig. 1 by the hydrolysis of acetyl- β -methylcholine, where a straight line is obtained when the logarithm of the optical density is plotted against the time.

In acid solutions the second term in equation (2) will be negligible compared with the first one, so that equation (2) can be expressed as

$$\log k = \log k_{\text{H}^+} - \text{pH} \quad (4)$$

In neutral and alkaline solutions equation (2) can be transformed into

$$\log k = \log k_{\text{OH}^-} - \text{p}K_w + \text{pH} \quad (5)$$

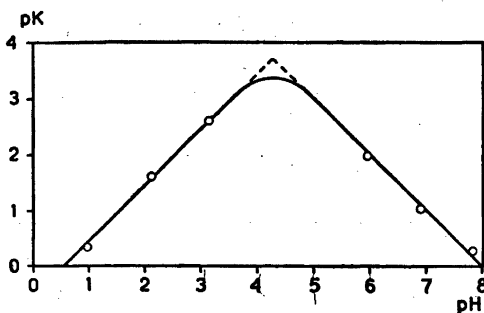


Fig. 2. Rate constants of the hydrolysis of acetyl- β -methylcholine at 75.0° C, plotted against the pH.

because k_{H^+} is much less than k_{OH^-} . Fig. 2 shows this to be valid within the experimental accuracy. From equations (4) and (5) the catalytic constants k_{H^+} and k_{OH^-} respectively were calculated in respect of the choline esters studied, and their values are given in Table 1.

Table 1. Catalytic constants k_{H^+} and k_{OH^-} for the hydrolysis of acetyl- β -methylcholine, propionylcholine, and butyrylcholine.

$t^\circ C$	Acetyl- β -methylcholine		Propionylcholine		Butyrylcholine	
	$k_{H^+} 10^3$ 1 · mole ⁻¹ ·sec. ⁻¹	k_{OH^-}	$k_{H^+} 10^3$ 1 · mole ⁻¹ ·sec. ⁻¹	k_{OH^-}	$k_{H^+} 10^3$ 1 · mole ⁻¹ ·sec. ⁻¹	k_{OH^-}
60.0	0.319	6.42	0.600	18.7	0.392	10.9
75.0	0.973	13.5	1.90	35.3	1.18	22.8
90.0	2.45	20.4	5.47	69.2	3.36	35.8

To obtain the activation energies of the acid catalyzed reactions the values of $\log k_{H^+}$ were plotted against $1/T$, as shown in Fig. 3. The lines were drawn according to the method of least squares, and the constants in Arrhenius' equation

$$\log k_{H^+} = A_{H^+} - B_{H^+}/T \quad (6)$$

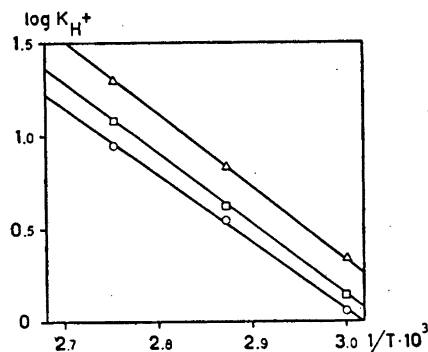


Fig. 3. Catalytic constants of the hydronium ion catalyzed hydrolysis of acetyl- β -methylcholine (O), propionylcholine (Δ), and butyrylcholine (\square) plotted against $1/T$.

were determined from the slope of the lines and from the intercepts. The corresponding constants for the base catalyzed reactions were determined in the same manner. The values of these constants and the activation energies, E , are given in Table 2.

Table 2. Values of the constants A and B in Arrhenius' equation and the activation energies, E , for the hydrolysis of acetyl- β -methyl-choline, propionylcholine, and butyrylcholine.

	A_{H^+}	B_{H^+}	E_{H^+} cal	A_{OH^-}	B_{OH^-}	E_{OH^-} cal
Acetyl- β -methylcholine	7.377	3 621	16 600	7.266	2 150	9 800
Propionylcholine	8.541	3 920	17 900	7.843	2 190	10 000
Butyrylcholine	7.933	3 780	17 300	7.653	2 203	10 100

In the acid hydrolysis of choline esters the reaction proceeds at approximately the same rate as in the acid hydrolysis of non-ionic esters. The length of the carbon-chain of the acid component and the branching of the alcohol component seem, however, to influence the rate of hydrolysis of choline esters less than that of ordinary esters. In alkaline hydrolysis minor changes in the structure of the alcohol component and acid component produce a greater effect on the reaction rate than in acid hydrolysis, which parallels that observed in ordinary ester hydrolysis. The rate of hydrolysis, however, is more rapid for choline esters than for non-ionic esters, which fact may be attributable to the greater affinity of the hydroxyl ion to the positively charged choline ester than to the uncharged ester.

SUMMARY

The kinetics of hydrolysis of the iodides of acetyl- β -methylcholine, propionylcholine, and butyrylcholine were studied in acid and alkaline buffer solutions at 60°, 75°, and 90° C; and the constants in Arrhenius' equation and the activation energies for these compounds were calculated.

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