

## Hydrolysis of Carboxylic Acid Esters of Thiocholine and its Analogues

### 1. Acid Hydrolysis

EDITH HEILBRONN

*Research Institute of National Defence, Dept. 1, Sundbyberg 4, Sweden*

The hydrolysis of thiocholine esters,  $\alpha$ -methylthiocholine esters and  $\beta$ -methylthiocholine esters was studied by a colorimetric method at pH-values 1.07, 1.65 and 2.20. First and second order rate constants are given. The temperature dependence of the hydrolysis was studied at 55°C, 75°C and 90°C at pH 1.2. The constants in Arrhenius' equation and activation energies are given.

The products of hydrolysis were studied by means of paper chromatography. Cleavage between the sulphur atom and the carbonyl group was found for all the esters investigated.  $R_F$ -values for choline and its analogues, choline esters, thiocholine and its analogues, thiocholine esters and the oxidation products of thiocholine and its analogues are given.

The results are discussed and a possible reaction mechanism for the hydrolysis of thioesters is given.

In the course of investigations, in this institute, concerning the relationship between the structure and cholinesterase affinity of substrates and inhibitors, series of carboxylic acid esters of thiocholine,  $\alpha$ -methylthiocholine and  $\beta$ -methylthiocholine were synthesized<sup>1,2</sup>.

Before a study of any enzyme kinetics or pharmacology with these compounds, it was considered necessary to study the dependence of their acid and alkaline hydrolysis on pH and temperature. Since up till now there has been relatively little known about the mechanism of hydrolysis of thioesters, it also seemed to be of interest to compare the hydrolysis of the thiocholine esters with the hydrolysis of the corresponding choline esters<sup>3</sup>.

Earlier data reported on the hydrolysis of thioesters seem mostly to refer to studies carried out in water-acetone or water-dioxan solutions. Morse and Tarbell<sup>4</sup> describe the hydrolysis in hydrochloric acid in 62 % acetone of allyl, benzyl and trityl thioacetates. They found cleavage between the carbonyl group and the sulphur atom with allyl and benzyl thioacetates, but cleavage between the alkyl group and the sulphur atom with trityl thioacetate. Noda

*et al.*<sup>5</sup>, Rylander and Tarbell<sup>6</sup> and Schaeffgen<sup>7</sup> have investigated the hydrolysis of several thioacetates, among them *n*-butyl, *isobutyl*, *n*-amyl and *isoamyl*, in hydrochloric acid. They found the hydrolysis to be first order with respect to ester concentration and hydronium ion concentration. However, Böhme and Schran<sup>8</sup>, who have studied the hydrolysis of some thioesters in water-dioxan and found a cleavage between acyl and sulphur, report catalysis by the hydronium ion which was not first order with respect to hydronium ion concentration, but dependent upon the type of acid used.

The dependence on pH and temperature of the hydrolysis of thiocholine and methylthiocholine esters reported in this paper was studied in acid buffer solutions. The products of hydrolysis were analyzed after hydrolysis in 0.1 M hydrochloric acid in a nitrogen atmosphere.

### EXPERIMENTAL

*Materials.* Thiocholine, thiocholine esters,  $\alpha$ -methylthiocholine esters and  $\beta$ -methylthiocholine esters were synthesized by Hansen<sup>1,2</sup>. Choline,  $\beta$ -methylcholine and acetyl- $\alpha$ -methylcholine were synthesized according to a method described by Tammelin<sup>9</sup> for the synthesis of acetylcholine and acetyl- $\beta$ -methylcholine. The esters were iodides or bromides. The thioacetic acid was commercial (Fluka A.G.).

*Paper chromatography.* Five solvents were used for the chromatography of the products of hydrolysis and their reference substances. The compositions of these solvents were

- 1) *n*-butanol saturated with water<sup>10</sup>,
- 2) *n*-butanol:ethanol:acetic acid:water (8:2:1:3)<sup>11</sup>,
- 3) *n*-propanol:1 M acetic acid (3:1)<sup>12</sup>,
- 4) diethylene glycol:*n*-butanol:water (1:4:1)<sup>13</sup>,
- 5) 2 M ammonium hydroxide:*isopropanol* (1:3)<sup>14</sup>.

Solvents 2–4 were used in descending technique, 1 and 5 in ascending technique. The filter paper used was Whatman No. 1. With the aid of a micropipette, 10–50  $\mu$ l of the solutions were placed in small portions on the filterpaper, which was dried between each addition. Duplicate spots were run. The reference substances were dissolved in water to determine their  $R_F$ -values and in 0.1 M hydrochloric acid, when they accompanied the hydrolysates. Mixtures of hydrolysate and reference substance were also run.

The esters were hydrolyzed in sealed tubes containing 0.1 M hydrochloric acid in a nitrogen atmosphere at 35°C for several days. Hydrolysates were analysed by chromatography. A hydrolysis where the solutions were kept at 80°C in a nitrogen atmosphere was followed by determining the amount of unhydrolyzed ester as iron-hydroxamate complex<sup>15</sup>.

The investigation of the spots was carried out by inserting the sheets into a chamber of iodine vapour in order to find the spots. Quaternary ammonium compounds were identified by spraying the chromatograms with dipicrylamine (DPA)<sup>16</sup> which gave yellow spots or by immersing the chromatograms in a solution of phosphomolybdic acid with subsequent reduction with stannous chloride (PMA)<sup>13</sup> which gave blue spots. Unhydrolyzed esters and thioacids were found as iron-hydroxamate complexes<sup>17</sup>. Thiols and also sometimes disulphides could be located with *N*-ethyl maleinimide (NEM)<sup>18</sup>, which gave red spots. This reagent reacted also with the unhydrolysed esters, which apparently were hydrolysed by the reagent. Hydroxyl groups usually gave yellow-coloured spots.

*Kinetic measurements.* Acid hydrolysis was studied in Clark and Lubs standard buffer solutions<sup>19</sup> at 55°C, 75°C and 90°C. The pH-values of the solutions were determined with a Radiometer pH-meter 23, type PHM 23C, and are given in Table 6. A saturated calomel electrode and a glass electrode, G302A, were used for measurements at high temperature. The instrument was standardised against a citrate buffer, pH 2.75  $\pm$  0.02 (55°C) (P-H Tamm, Svenska Finkemikalier).

Table 1. Chromatogram following oxidation of thiocholine, acetyl- $\alpha$ -methylthiocholine and acetyl- $\beta$ -methylthiocholine. Solvent No. 4. Solvents are described in the experimental part.

Time (h) after oxidation start	Oxidised compound								
	Thiocholine			Acetyl- $\alpha$ -methylthiocholine			Acetyl- $\beta$ -methylthiocholine		
	$R_F$	colour with	indicating	$R_F$	colour with	indicating	$R_F$	colour with	indicating
0	0.60 (0.22)	PMA, NEM PMA, NEM	thiocholine (oxidised thiocholine)	0.72	PMA, NEM	(acetyl-) $\alpha$ - methyl- thiocholine	0.62	PMA, NEM	(acetyl-) $\beta$ - methyl- thiocholine
4	0.60 (0.22)	PMA, NEM PMA, NEM	thiocholine (oxidised thiocholine)	0.72  (0.24)	PMA, NEM  PMA	(acetyl-) $\alpha$ - methyl- thiocholine (oxidised $\alpha$ -methyl- thiocholine)	0.62  (0.29)	PMA, NEM  PMA	(acetyl-) $\beta$ - methyl- thiocholine (oxidised $\beta$ -methyl- thiocholine)
22	0.22	PMA, NEM	oxidised thiocholine	0.24	PMA (NEM)	oxidised $\alpha$ -methyl- thiocholine	0.29	PMA	oxidised $\beta$ -methyl- thiocholine
60	0.22	PMA, NEM	oxidised thiocholine	0.24	PMA	oxidised $\alpha$ -methyl- thiocholine	0.29	PMA	oxidised $\beta$ -methyl- thiocholine

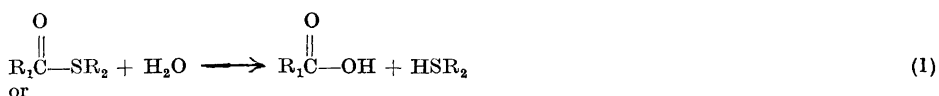
$4.0 \times 10^{-3}$  M solutions of the compounds were hydrolysed in a thermostat. The pH of the solution was measured at the beginning and at the end of each kinetic experiment. The ester concentration in solutions was determined as iron-hydroxamate complex<sup>15</sup> and measured with a Beckman B spectrophotometer at 540  $m\mu$ .

*Preparation of disulphide compounds.* Bis (trimethyl ammonium ethyl) disulphide (oxidised thiocholine): 100 mg of thiocholine iodide were dissolved in 25 ml of 48 % hydrogen bromide,  $d$  1.49. The solution was refluxed at the boiling point and a stream of oxygen was led in. The progress of the oxidation was followed with hydroxamate- $\text{FeCl}_3$  and also by chromatography. In this way it was made clear which spot was the thiol and which was the disulphide. After 60 h all the thiocholine was oxidised. Bis(trimethyl ammonium 2-methyl ethyl) disulphide, oxidised  $\alpha$ -methylthiocholine, and bis(trimethyl ammonium 1-methyl ethyl) disulphide, oxidised  $\beta$ -methylthiocholine, were prepared in the same way, but here the starting material was the corresponding acetyl ester. Chromatograms are shown in Table 1.

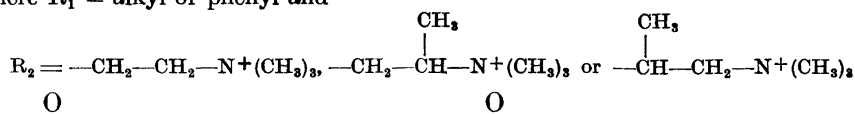
## RESULTS

### Products of hydrolysis

The hydrolysis of thiocholine and its analogues can be expected to proceed according to one of the following reaction schemes:



where  $R_1$  = alkyl or phenyl and



As  $R_1\overset{\text{O}}{\parallel}{\text{C}}\text{---SH}$  in acid medium splits into  $R_1\overset{\text{O}}{\parallel}{\text{C}}\text{---OH}$  and  $\text{H}_2\text{S}$ , but  $\text{HSR}_2\text{N}^+(\text{CH}_3)_3$  probably is stable at least at moderate temperatures, the hydrolysates were analysed with respect to choline and thiocholine or their analogues.

Table 2.  $R_F$ -values for several quaternary ammonium compounds and thioacetic acid in 5 different solvents, described in the experimental part.

Compound	$R_F$ in solvent					Spots developed with
	1	2	3	4	5	
Choline chloride	0.09	0.25	0.40	0.39	—	$I_2$ , PMA, DPA
Choline bromide	0.12	0.27	0.43	0.41	—	$I_2$ , PMA, DPA
Choline iodide	0.18	0.34	0.52	0.51	—	$I_2$ , PMA, DPA
$\beta$ -Methylcholine iodide	0.27	0.44	0.61	0.60	—	$I_2$ , PMA, DPA, FeH
Acetylcholine iodide	0.22	0.39	0.56	0.56	—	$I_2$ , PMA, DPA, FeH
Acetyl- $\alpha$ -methylcholine iodide	0.30	0.45	0.60	0.60	—	$I_2$ , PMA, DPA, FeH
Acetyl- $\beta$ -methylcholine iodide	0.35	0.47	0.66	0.67	—	$I_2$ , PMA, DPA, FeH
Thiocholine iodide	0.25	0.43	0.55	0.62	—	$I_2$ , PMA, DPA, Nem
Acetylthiocholine bromide	0.24	0.41	0.52	0.60	—	$I_2$ , PMA, DPA, FeH, Nem
Acetylthiocholine iodide	0.28	0.43	0.59	0.65	—	$I_2$ , PMA, DPA, FeH, Nem
Acetyl- $\alpha$ -methylthiocholine bromide	0.32	0.50	0.60	0.71	—	$I_2$ , PMA, DPA, FeH, Nem
Acetyl- $\alpha$ -methylthiocholine iodide	—	—	0.70	0.78	—	$I_2$ , PMA, DPA, FeH, Nem
Acetyl- $\beta$ -methylthiocholine bromide	0.29	0.47	0.61	0.62	—	$I_2$ , PMA, DPA, FeH, Nem
Acetyl- $\beta$ -methylthiocholine iodide	0.30	0.47	0.64	0.66	—	$I_2$ , PMA, DPA, FeH, Nem
Valeryl- $\beta$ -methylthiocholine iodide	—	0.72	—	—	—	$I_2$ , PMA, DPA, FeH, Nem
Bis(tri-methyl ammonium ethyl) disulphide, oxidized thiocholine	0.04	0.19	0.26	0.23	—	$I_2$ , PMA, DPA, (Nem)
Bis(tri-methyl ammonium 1-methyl ethyl) disulphide, oxidized $\beta$ -methylthiocholine	0.08	0.20	0.31	0.29	—	$I_2$ , PMA, DPA, (Nem)
Bis(tri-methyl ammonium 2-methyl ethyl) disulphide, oxidised $\alpha$ -methylthiocholine	{0.06 0.17}	{0.18 0.34}	0.33	{0.32 0.24}	—	$I_2$ , PMA, DPA, (Nem)
Thioacetic acid	—	—	0.92	—	0.58	Nem, FeH

Table 3. Chromatogram showing hydrolysis of acetylthiocholine at 80°C in 0.1 M HCl.

Time in h	$R_F$	Colour with	Indicating
0	0.66 (0.62)	PMA, Nem, FeH PMA, Nem	acetylthiocholine (thiocholine)
0.1	0.66 (0.62)	PMA, Nem, FeH PMA, Nem	acetylthiocholine (thiocholine)
1.0	0.66 0.62	PMA, Nem, FeH PMA, Nem	acetylthiocholine thiocholine
2.5	0.66 0.62	PMA, Nem, FeH PMA, Nem	acetylthiocholine thiocholine
4.5	(0.66) 0.62 0.21	PMA, Nem, FeH PMA, Nem PMA	(acetylthiocholine) thiocholine oxidised thiocholine
22	0.62 0.21	PMA, Nem PMA, Nem	thiocholine oxidised thiocholine
28	0.62 0.21	PMA, Nem PMA, Nem	thiocholine oxidised thiocholine
48	0.62 0.21	PMA, Nem PMA, Nem	thiocholine oxidised thiocholine

Before starting the chromatograms of the products of hydrolysis, the  $R_F$ -values for the expected reference substances were determined. Values are given in Table 2. Observed variation of  $R_F$ -values in different runs was about  $\pm 10\%$ . It is seen that  $R_F$ -values for thioesters increase in the order chlorides, bromides, iodides. This has also been observed for quaternary ammonium compounds by Sheppars *et al.*<sup>20</sup>.

A chromatogram following hydrolysis of acetylthiocholine iodide is shown in Table 3. Blue colour with phosphomolybdic acid and stannous chloride

Table 4. Chromatogram of hydrolysate of acetyl- $\beta$ -methylthiocholine after hydrolysis in 0.1 M HCl in sealed tubes filled with nitrogen.

Solvent	$R_F$	Colour with	Indicating
1	0.27 (0.04)	PMA, Nem PMA	$\beta$ -methylthiocholine (oxidised $\beta$ -methyl- thiocholine)
2	0.39 (0.15)	PMA, Nem PMA	$\beta$ -methylthiocholine (oxidised $\beta$ -methyl- thiocholine)
3	0.52 (0.32)	PMA, Nem PMA	$\beta$ -methylthiocholine (oxidised $\beta$ -methyl- thiocholine)
4	0.61 (0.31)	PMA, Nem	$\beta$ -methylthiocholine (oxidised $\beta$ -methyl- thiocholine)

**Table 5.** Chromatogram of hydrolysate of acetyl- $\alpha$ -methylthiocholine after hydrolysis in 0.1 M HCl in sealed tubes filled with nitrogen.

Solvent	$R_F$	Colour with	Indicating
1	0.31 (0.09 + 0.17)	PMA, Nem PMA	$\alpha$ -methylthiocholine (oxidised $\alpha$ -methyl- thiocholine)
2	0.45 (0.21 + 0.34)	PMA, Nem PMA	$\alpha$ -methylthiocholine (oxidised $\alpha$ -methyl- thiocholine)
3	0.53 (0.45)	PMA, Nem PMA	$\alpha$ -methylthiocholine (oxidised $\alpha$ -methyl- thiocholine)
4	0.64 (0.31)	PMA, Nem PMA	$\alpha$ -methylthiocholine (oxidised $\alpha$ -methyl- thiocholine)

(PMA) indicates a quaternary ammonium group or a tertiary amino group. For the esters analysed the identification of a quaternary ammonium group was accepted. The iron-hydroxamate complex (FeH) indicates an ester linkage and a colour reaction with N-ethyl-maleinimide (NEM) indicates a thio group. However, in a few cases red colour with NEM was observed also with the disulphide compounds. The colour of the spots and their  $R_F$ -values identify them as acetylthiocholine ( $R_F$  0.66), thiocholine ( $R_F$  0.62) and probably oxidised thiocholine ( $R_F$  0.21).

The hydrolysates of acetyl- $\beta$ -methylthiocholine bromide gave, after 3 days at 55°C,  $\beta$ -methylthiocholine and very small amounts of oxidised  $\beta$ -methylthiocholine, as seen in Table 4. In the same way hydrolysis of acetyl- $\alpha$ -methylthiocholine bromide was shown to give  $\alpha$ -methylthiocholine and small amounts of oxidised  $\alpha$ -methyl-thiocholine, as seen in Table 5. For some unknown reason oxidised  $\alpha$ -methyl-thiocholine often gave two spots.

Thus chromatograms of the products of acid hydrolysis of acetylthiocholine, acetyl- $\alpha$ -methylthiocholine and acetyl- $\beta$ -methylthiocholine show that, for all three types of esters, cleavage occurs between the carbonyl group and the sulphur atom. This was also shown to be true for the other thioesters analysed, including the benzoyl thioesters.

### Rate of hydrolysis

The rate of hydrolysis for thiocholine esters and their analogues was determined in buffer solutions at pH—values 1.07, 1.65 and 2.20 at 55°C. The ionic strengths ( $\mu$ ) of the solutions were estimated to be 0.142 at pH 1.07, 0.077 at pH 1.65 and 0.057 at pH 2.20. Straight lines were obtained when the logarithm of the ester concentration at constant pH was plotted against time as is seen

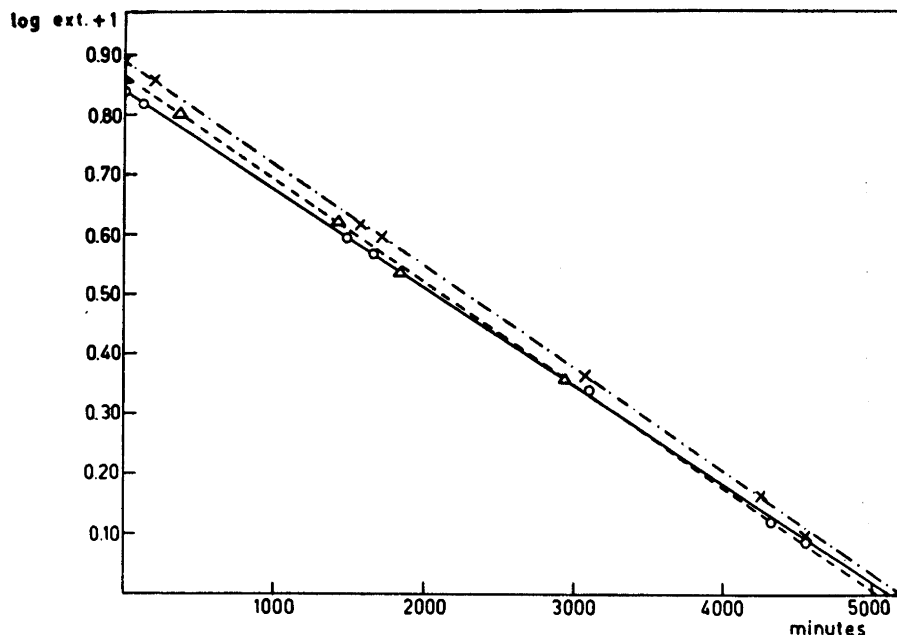


Fig. 1. Hydrolysis at pH 1.07. Logarithm of ester concentration, expressed as logarithm of optical density of iron-hydroxamate complex, plotted against time.  $\times$  = acetylthiocholine,  $\circ$  = acetyl- $\alpha$ -methylthiocholine,  $\Delta$  = acetyl- $\beta$ -methylthiocholine.

in Fig. 1, indicating a first order reaction. First order rate constants in minutes were calculated according to equation

$$k_1 = \frac{1}{t} \ln \frac{c_0}{c}$$

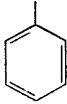
where  $c_0$  and  $c$  are the optical densities at zero time and time  $t$ , respectively. It was shown earlier<sup>15</sup> that Lambert-Beer's law is obeyed. Second order rate constants in  $l \cdot \text{mole}^{-1} \cdot \text{min}^{-1}$  were calculated according to equation

$$k_2 \cdot a_{\text{H}^+} = k_1$$

as the catalysis by hydroxyl ions can be neglected at the pH-values investigated. Second order rate constants at ionic strength = 0 were not calculated, as Nilsson (Olered)<sup>21,22</sup> has shown that the ionic strength does not seem to influence the rate of the acid hydrolysis of choline esters.

Rate constants given in Table 6 show that the rate is proportional to hydrogen ion activity with a slight tendency to increase at the highest pH used.

Table 6. Pseudo first order rate constants and second order rate constants for several carboxylic acid esters of thiocholine,  $\alpha$ -methylthiocholine and  $\beta$ -methylthiocholine, determined at pH 1-2.2,  $t = 55^\circ\text{C}$ .

$R_1$	$R_2$	$-\text{CH}_2-\text{CH}_2-\text{N}^+(\text{CH}_3)_3$			$-\text{CH}_3-\text{CH}-\text{N}^+(\text{CH}_3)_3$   $\text{CH}_3$			$-\text{CH}-\text{CH}_2-\text{N}^+(\text{CH}_3)_3$   $\text{CH}_3$		
$\text{CH}_3-$	pH	1.07	1.65	2.22	1.07	1.65	2.20	1.07	1.65	2.20
	$k_1 \text{ min}^{-1} \times 10^4$	4.04	1.18	0.407	3.09	0.805	0.305	3.95	1.03	0.400
	$k_{21} \times \text{mole}^{-1} \times \text{min}^{-1} \times 10^3$	4.8	5.2	6.8	3.5	3.6	4.8	4.7	4.6	6.3
$\text{CH}_3\text{CH}_2-$	pH	1.03	1.65	2.23	1.03	1.62	2.22	1.00	1.60	2.26
	$k_1 \text{ min}^{-1} \times 10^4$	3.45	0.776	0.313	2.84	0.810	0.190	3.49	0.922	0.189
	$k_{21} \times \text{mole}^{-1} \times \text{min}^{-1} \times 10^3$	3.7	3.5	5.3	3.0	3.4	3.2	3.5	3.6	3.4
$\text{CH}_3\text{CH}_2\text{CH}_2-$	pH	1.05	1.62	2.25	1.05	1.62	2.21	1.00	1.65	2.26
	$k_1 \text{ min}^{-1} \times 10^4$	1.99	0.486	0.204	1.38	0.503	0.103	2.25	0.410	0.147
	$k_{21} \times \text{mole}^{-1} \times \text{min}^{-1} \times 10^3$	2.2	2.0	3.6	1.6	2.0	1.7	2.3	1.8	2.7
$\begin{array}{c} \text{CH}_3 \\ \diagdown \\ \text{C} \\ \diagup \\ \text{CH}_3 \end{array} \text{CH}_2-$	pH	1.07	1.65	2.20	—	—	—	—	—	—
	$k_1 \text{ min}^{-1} \times 10^4$	1.55	0.422	0.131	—	—	—	—	—	—
	$k_{21} \times \text{mole}^{-1} \times \text{min}^{-1} \times 10^3$	1.8	1.9	2.1	—	—	—	—	—	—
$\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2-$	pH	1.05	1.65	2.25	1.05	1.62	2.21	1.00	1.65	2.26
	$k_1 \text{ min}^{-1} \times 10^4$	1.96	0.473	0.200	1.38	0.501	0.154	1.96	0.434	0.184
	$k_{21} \times \text{mole}^{-1} \times \text{min}^{-1} \times 10^3$	2.2	2.1	3.6	1.5	2.1	2.5	2.0	2.0	3.4
	pH	1.05	—	—	1.07	—	—	1.07	—	—
	$k_1 \text{ min}^{-1} \times 10^4$	0.138	—	—	0.072	—	—	0.148	—	—
	$k_{21} \times \text{mole}^{-1} \times \text{min}^{-1} \times 10^3$	0.2	—	—	0.1	—	—	0.2	—	—



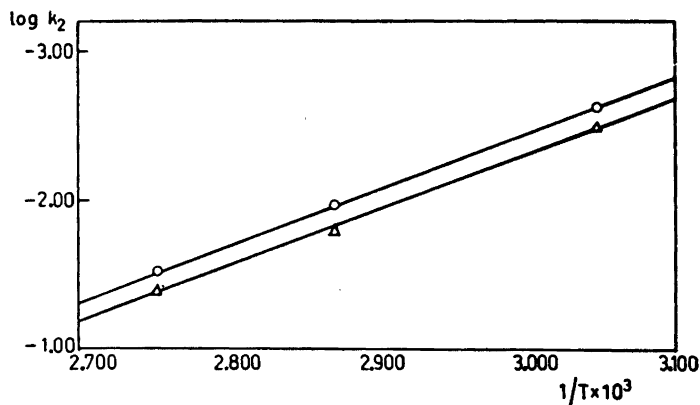


Fig. 2. Temperature dependence of hydrolysis. Second order rate constants in  $l \times \text{mole}^{-1} \times \text{min}^{-1}$  plotted against  $1/T$ . Lines calculated by method of least squares.  $\Delta$  = acetylthiocholine,  $\circ$  = acetyl- $\alpha$ -methylthiocholine.

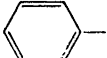
### Temperature dependence

Temperature dependence of the acid hydrolysis was determined for four esters of each of the thiols at pH 1.2 at 55°C, 75°C and 90°C. Difficulties in the determination of the exact pH at the high temperatures which were necessary to speed up hydrolysis, influence the accuracy of the calculated values. The logarithms for second order rate constants in  $l \cdot \text{mole}^{-1} \cdot \text{min}^{-1}$  were calculated as described earlier and plotted against  $1/T$  as seen in Fig 2. The line

$$\log k_2 = A_{H^+} + B_{H^+}/T$$

representing Arrhenius' equation, was calculated by means of the method of least squares, and the activation energies were calculated. The logarithm for the collision factor, PZ, is equal to  $A_{H^+} \cdot \text{Arrhenius' constants}$  and activation energies are shown in Table 7.

Table 7. Constants in Arrhenius' equation and activation energies for several carboxylic acid esters of thiocholine,  $\alpha$ -methylthiocholine and  $\beta$ -methylthiocholine at pH 1.2.

R <sub>1</sub> \ R <sub>2</sub>	-CH <sub>2</sub> -CH <sub>2</sub> -N <sup>+</sup> (CH <sub>3</sub> ) <sub>3</sub>			-CH <sub>2</sub> -CH(CH <sub>3</sub> )-N <sup>+</sup> (CH <sub>3</sub> ) <sub>3</sub>			-CH(CH <sub>3</sub> )-CH <sub>2</sub> -N <sup>+</sup> (CH <sub>3</sub> ) <sub>3</sub>		
	A <sub>H<sup>+</sup></sub>	B <sub>H<sup>+</sup></sub>	E kcal	A <sub>H<sup>+</sup></sub>	B <sub>H<sup>+</sup></sub>	E kcal	A <sub>H<sup>+</sup></sub>	B <sub>H<sup>+</sup></sub>	E kcal
CH <sub>3</sub> -	8.8	3 590	16.4	8.7	3 440	15.7	8.5	3 610	16.5
CH <sub>3</sub> -CH <sub>2</sub> -CH <sub>2</sub> -	8.8	3 710	17.0	9.4	3 980	18.2	8.6	3 660	16.7
$\begin{array}{l} \text{CH}_3 \\ \diagdown \\ \text{CH}_2- \\ \diagup \\ \text{CH}_3 \end{array}$	8.8	3 740	17.1	9.5	4 130	18.9	—	—	—
	9.3	4 270	19.5	9.6	4 450	20.4	9.4	4 370	20.0

## DISCUSSION

The rate of hydrolysis of the thioesters studied is rather low compared with the rate of the corresponding choline esters, determined in the same medium by Larsson<sup>3</sup>. The thiocholine esters hydrolyse at 75°C about 3 times slower than their choline analogues. The same phenomenon has been found by other authors<sup>7</sup>, who have compared the rate of hydrolysis of thioesters with that of the corresponding oxygen esters. Sulphur is considered to be less negative than oxygen, which results in a less positive carbon in the C—S bond than in the C—O bond. This would retard the water attack on the carbon, supposed to be the rate determining step in acid hydrolysis. Even hydrogen ion attack on the sulphur would be slower than on the oxygen.

A comparison of activation energies found for thiocholine esters with those found for choline esters<sup>3</sup> shows no difference in these values, indicating that the two types of ester may hydrolyse by the same mechanism.

The effect of the substituent on the thiol on the rate of hydrolysis is not great. The esters of the unsubstituted thiocholines hydrolyse fastest, immediately followed by  $\beta$ -methyl substituted esters. The  $\alpha$ -methyl substituted esters show the slowest rate of hydrolysis. No steric effect of the  $\beta$ -methyl group could be observed. The reason for this may be the length of the C—S—C bond, according to Pauling 2.58 Å. A methyl group in the  $\beta$ -position to the ammonium group in the thiol would be far enough away from the attacked carbon atom to have little chance of hindering the water molecule from reaching the carbon. Also the small hydrogen ion would easily reach the sulphur atom for an attack in the second step of the hydrolysis.

It is more difficult to explain the small but obvious retardation of hydrolysis found for the  $\alpha$ -substituted esters. Several anomalies in the behaviour of these esters were observed. They were found to be the least water-soluble esters and they showed a different behaviour in chromatography from that of the choline esters. Thus it was found that the  $R_F$ -values for choline esters increase in the order unsubstituted,  $\alpha$ -methyl substituted,  $\beta$ -methyl substituted ester, but for the thiocholine esters the order was found to be unsubstituted,  $\beta$ -methyl substituted,  $\alpha$ -methyl substituted ester. This indicates that  $\alpha$ -methyl substituted thiocholine esters have a lower affinity for water than the other thioesters and would be in agreement with the observed lower rate of hydrolysis.

The substituent on the carboxy group of the thioesters seems to be important for the rate of hydrolysis of the esters. The rate decreases as the carbon chain grows longer, and bulky substituents retard hydrolysis more than their straight analogues. Thus the rate of hydrolysis for the *isobutyryl* substituted thioester is lower than for *n*-butyryl substituted thioester. It is remarkable that the benzoyl thioester is practically not hydrolysed at all at 55°C. The effect of these substituents may be explained as a steric as well as an inductive and mesomeric (*e.g.* phenyl) effect. If the attack of water at the carbonyl carbon is the rate determining step, bulky substituents on this atom should retard the hydrolysis and thus have a greater influence upon the reaction rate than substituents at the thiol, which is in agreement with the experimental results. The electron releasing effect of the substituents increases with the length

