

# Hydrolysis of Carboxylic Acid Esters of Thiocholine and its Analogues

## 2. Alkaline Hydrolysis

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The hydrolysis of thiocholine esters,  $\alpha$ -methylthiocholine esters and  $\beta$ -methylthiocholine esters was studied with an automatic recording titrator at pH values 9.00, 9.50, 9.75 and 10.00 at 35°C and with a colorimetric method at pH 6.0, 7.0 and 8.0 at 55°C. First and second order rate constants are given.

The dependence of the hydrolysis rate on temperature was studied at 25°C, 30°C, and 35°C at pH 10.00. Arrhenius' constants and activation energies are given. The products of hydrolysis were studied by means of paper chromatography and paper ionophoresis and found to be fairly complex. Cleavage between the sulphur atom and the carbonyl group was found.

The hydrolysis of acetylcholine, acetyl- $\alpha$ -methylcholine and acetyl- $\beta$ -methylcholine was studied with an automatic titrator at pH 9.00, 9.50 and 10.00 at 35°C. First and second order rate constants, Arrhenius' constants and activation energies are given. The results and possible reaction mechanisms are discussed.

Previous studies on the alkaline hydrolysis of thioesters<sup>1-7</sup> refer mostly to hydrolysis in various amounts of sodium hydroxide. Most experiments were carried out under conditions, to which the mathematical treatment of second order kinetics could be applied. Some experiments<sup>3, 5, 6</sup> were, however, carried out in large excess of hydroxyl ion, thus keeping the pH approximately constant. The authors have found agreement with the theory for second order reactions up to 60—80 % completion of hydrolysis. Cleavage between acyl and sulphur was found in all cases. However, Morse and Tarbell<sup>4</sup> found after hydrolysis of tritylthioacetate certain amounts of triphenylcarbinol, but this seems to be a secondary product, as mainly material of disulphide type, among others probably dinitryl disulphide, was obtained.

The only papers known to the present author and treating hydrolysis of thioesters at lower, exactly defined pH-values are by Koningsberger and Overbeek<sup>8-11</sup>. They studied the hydrolysis of ethyl thioacetate between pH 8

and 12 in 0.2 M borate buffer and found that the rate of hydrolysis was directly proportional to the hydroxyl ion concentration up to pH 8.6, as is usually the case with oxygen esters. Above this pH the second order rate constant started to decrease rapidly down to about 10 % of its original value, thereafter proportionality to hydroxyl ion concentration was again observed. They explained this observation by assuming two competitive reactions, a hydroxyl ion catalysed hydrolysis prevailing at high pH and another type of hydrolysis, prevailing at lower pH. Several ways were proposed for the latter type of hydrolysis.

In the preceding paper<sup>12</sup> the acid hydrolysis of carboxylic acid esters of thiocholine and two methylthiocholines is described. The study of the alkaline hydrolysis of these compounds was started with the methods<sup>12</sup> already described. However, complications occurred when the hydroxamate-FeCl<sub>3</sub> method<sup>13</sup> was used for kinetic measurement at higher pH-values and so it was decided to use an automatic recording titrator<sup>14</sup>. The dependence of the reaction rate on pH and temperature was also measured for some corresponding choline esters.

#### EXPERIMENTAL

*Materials.* The same preparations as for the study on acid hydrolysis<sup>12</sup> were used.

*Paper chromatography.* Solvents, paper and technique as described earlier<sup>12</sup>.

The esters were hydrolysed in excess of 0.1 M sodium hydroxide in an atmosphere of nitrogen previous to chromatography with procedure as described earlier<sup>12</sup>. Hydrolysates from titrations at pH 9.0 were also subject to chromatographic analysis. The hydrolysates were acidified to prevent oxidation during chromatography.

*High voltage paper ionophoresis.* Hydrolysates from kinetic measurements were used. Paper ionophoresis was carried out with an apparatus according to Foster<sup>15</sup> on Whatman paper No. 1 at a voltage of 2 000 V, 40 V/cm. A 0.1 M borate buffer solution of pH 10.0 was used. Spots were placed on dry paper with a micropipette. After being air dried the papers were immersed in the buffer solution. Excess buffer solution was removed with filter papers. After ionophoresis the papers were air dried and the spots were developed as described earlier for paper chromatography<sup>12</sup>.

*Kinetic measurements.* The alkaline hydrolysis was at first studied in modified Clark and Lubs standard buffer solutions<sup>16</sup> at 55°C. The pH-values of the solutions were determined against a standard buffer of pH 6.95 ± 0.02 (55°C) (P-H Tamm) as described earlier<sup>12</sup>.

Automatic titration at constant pH was carried out at constant ionic strength in 0.1 M potassium chloride. The titrator was a 0.098 M solution of sodium hydroxide. The glass electrode used was a Radiometer G 202B and the reference electrode was a saturated calomel electrode, Radiometer K 300. In most experiments  $2.77 \times 10^{-5}$  moles of thiocholine esters were titrated. The same method was used for the determination of the rate constants of some corresponding choline esters.  $5.54 \times 10^{-5}$  moles of these compounds were titrated.

In a few experiments at pH 9.00 and 35°C the titrator was used to keep the pH constant and the amount of unhydrolysed ester was determined as iron-hydroxamate complex<sup>13</sup> in a Beckman apparatus at 540 m $\mu$ . In these experiments the concentration of the esters was  $2.77 \times 10^{-4}$  M and the titrator was 0.502 M sodium hydroxide.



Table 1. Chromatograms after hydrolysis of acetylthiocholine and acetyl- $\alpha$ -methylthiocholine at 35°C in excess of alkali and after hydrolysis of valeryl- $\beta$ -methylthiocholine at pH 9.00 and 35°C.

| Solvent         | $R_F$  | Colour with | Indicating                               |
|-----------------|--------|-------------|--|
| 1 <sup>23</sup> | 0.18   | PMA, (NEM)  | ?<br>thiocholine                         |
|                 | 0.27   | PMA, NEM    |  |
| 2 <sup>24</sup> | 0.39   | PMA         | ?<br>thiocholine                         |
|                 | 0.45   | PMA, NEM    |  |
| 3 <sup>25</sup> | 0.52   | PMA         | ?<br>thiocholine                         |
|                 | 0.58   | PMA, NEM    |  |
| 4 <sup>26</sup> | 0.37   | PMA, (NEM)  | ?<br>thiocholine                         |
|                 | 0.60   | PMA, NEM    |  |
| 3 <sup>25</sup> | 0.34   | PMA         | oxidised $\alpha$ -methylthiocholine     |
|                 | 0.42   | PMA, (NEM)  | ?<br>$\alpha$ -methylthiocholine         |
|                 | 0.61   | PMA, NEM    |  |
| 2 <sup>24</sup> | 0.48   | PMA, NEM    | $\beta$ -methylthiocholine               |
|                 | 0.65   | PMA, (NEM)  | ?<br>valeryl- $\beta$ -methylthiocholine |
|                 | (0.72) | PMA, NEM    |  |

Table 2. Chromatogram of solution of 100 mg of thiocholine after 4 days in 0.1 M sodium hydroxide at 35°C.

| Solvent         | $R_F$ | Colour with | Indicating           |
|-----------------|-------|-------------|----------------------|
| 1 <sup>24</sup> | 0.03  | PMA         | oxidised thiocholine |
|                 | 0.18  | PMA         | choline ?            |
|                 | 0.27  | PMA, NEM    | thiocholine          |
|                 | 0.45  | PMA, NEM    | ?                    |
| 2 <sup>25</sup> | 0.26  | PMA         | choline ?            |
|                 | 0.43  | PMA, NEM    | thiocholine          |
|                 | 0.55  | PMA, NEM    | ?                    |
| 3 <sup>26</sup> | 0.44  | PMA         | choline ?            |
|                 | 0.57  | PMA, NEM    | thiocholine          |
|                 | 0.69  | PMA, NEM    | ?                    |
| 4 <sup>27</sup> | 0.36  | PMA         | choline ?            |
|                 | 0.60  | PMA, NEM    | thiocholine          |
|                 | 0.76  | PMA, NEM    | ?                    |

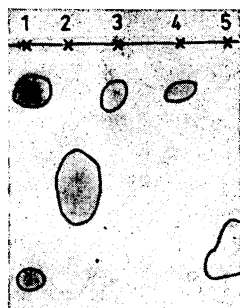


Fig. 1. Ionophoretogram of hydrolysate of valeryl- $\beta$ -methylthiocholine. 1 = mixture of  $\beta$ -methylthiocholine and oxidised  $\beta$ -methylthiocholine (colour with NEM, PMA), 2 = valeryl- $\beta$ -methylthiocholine (NEM, PMA), 3 and 4 = hydrolysate (NEM, PMA), 5 =  $\beta$ -methylcholine (PMA).

To determine the products of hydrolysis it was decided to use high voltage paper ionophoresis at a pH above the  $pK'_a$  of thiocholine and methylthiocholine. The  $pK'_a$  of thiocholine was found to be  $\sim 7.7$  by titration at ionic strength  $\mu = 0.1$  with an automatic recording titrator<sup>14</sup>. The values for the other two compounds had to be approximated from the  $pK'_a$ -values of analogous dimethylamino compounds. They were found by Hansen<sup>17</sup> to be 7.84 for dimethylamino ethanthiol, 7.59 for dimethylamino- $\alpha$ -methylethanthiol and 7.62 for dimethylamino- $\beta$ -methylethanthiol.

It was therefore decided that a borate buffer solution of pH 10 should be used. At this pH it can be expected that thiocholine and its analogues will be dissociated and, owing to their state of "Zwitterion",  $^-SRN^+(CH_3)_3$ , will not move far from the starting point of the ionophoresis, but choline and its analogues, which are not dissociated at this pH, will move rapidly towards the negative pole because of their positive charge,  $HORN^+(CH_3)_3$ . Secondary oxidation products, having two positive charges, can also be expected to move rapidly towards the negative pole.

The results of the ionophoresis of the products of hydrolysis are given for valeryl- $\beta$ -methylthiocholine in Fig. 1. It is clearly seen that no  $\beta$ -methylcholine is obtained, whereas  $\beta$ -methylthiocholine is. Hydrolysates of thiocholine esters and  $\alpha$ -methylthiocholine esters showed spots indicating thiocholine and  $\alpha$ -methylthiocholine, respectively. A spot of unknown origin was sometimes obtained between spots of unhydrolysed ester and thiol. It thus seems that cleavage between the sulphur atom and the carbonyl group occurs in alkaline hydrolysis, but that the products are somewhat complex even when oxidation is prevented during hydrolysis.

#### Rate of hydrolysis

The rate of hydrolysis for choline and thiocholine esters and their analogues was determined at the pH-values 9.00, 9.50, 9.75 (only  $\alpha$ -methyl and  $\beta$ -methylthiocholine esters) and 10.00 at 35°C and at constant ionic strength,  $\mu = 0.1$ . The pseudo first order rate constant in  $\text{min}^{-1}$  was calculated according to the method of Guggenheim<sup>19</sup> except for  $\beta$ -methylthiocholine esters at pH 9.00. Second order rate constants were calculated according to the equation

$$k_2 \cdot a_{OH^-} = k_1 \quad (3)$$

Table 3. Pseudo first order rate constants, second order rate constants, and second order rate constants at ionic strength = 0 for acetylcholine, acetyl- $\alpha$ -methylcholine and acetyl- $\beta$ -methylcholine.

| 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> |      |       |
|-------------------|--|---|------|-------|---|------|-------|---|------|-------|
| R <sub>1</sub>    | pH   | 9.00  | 9.50 | 10.00 | 9.00  | 9.50 | 10.00 | 9.00  | 9.50 | 10.00 |
|                   | $k_1 \text{ min}^{-1} \times 10^3$                                   | 4.75  | 13.9 | 46.9  | 4.05  | 12.4 | 41.3  | 1.10  | 3.40 | 11.4  |
| CH <sub>3</sub> - | $k_2 \text{ l} \times \text{mole}^{-1} \times \text{min}^{-1}$       | 220 ± 8   |      |       | 193 ± 5   |      |       | 52.9 ± 1.7  |      |       |
|                   | $k_2^\circ \text{ l} \times \text{mole}^{-1} \times \text{min}^{-1}$ | 302 ± 12  |      |       | 269 ± 7   |      |       | 72.4 ± 3.2  |      |       |

and  $k_2^\circ$  was calculated according to

$$\log k_2 + \log f_{\text{OH}^-} = \log k_2^\circ + z_a z_b \sqrt{\mu} - 0.6 z_a z_b \mu \quad (4)$$

where  $z_a$  and  $z_b$  are the charges of the reacting ions and 0.6 is a constant found and used by Olered<sup>20</sup> and shown by him to be valid for acetylcholine and some of its analogues. The value for  $\log f_{\text{OH}^-}$  used in the equations was estimated to be -0.12. The error in  $k_2$ , given in Table 3, is a maximum error.

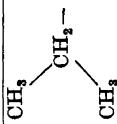
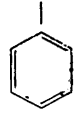
*Choline esters.* The constant values of  $k_2$  obtained for choline esters and their analogues and given in Table 3, show that the rate of hydrolysis of these esters depends only upon hydroxyl ion activity. The use of eqn. (3) is thus justified for these esters.

*Thiocholine esters.* Table 4 shows  $k_2$  for thiocholine esters and their analogues. It is seen that  $k_2$  for thiocholine esters is practically constant, though there seems to be a very slight tendency towards a higher value at lower pH. The logarithm of ester concentration, calculated from the amount of sodium hydroxide consumed, plotted against time, shows a straight line, indicating a pseudo first order reaction. Thus it is justified to use eqn. (3) also for these esters.

*$\alpha$ -Methylthiocholine esters.* The " $k_2$ " values found for  $\alpha$ -methylthiocholine esters, given in Table 4, show a tendency to decrease at lower pH. Plots of  $\log c$  against time show no perfectly straight lines. It is questionable if the method of Guggenheim can be used for calculations of these reaction rates.

*$\beta$ -Methylthiocholine esters.* The " $k_2$ " values for  $\beta$ -methylthiocholine esters increase with decreasing pH. A check of the assumed pseudo first order rate of hydrolysis at constant pH, again done by plotting  $\log c$  against time (Fig. 2), showed no straight line for any  $\beta$ -methylthiocholine ester at pH below 9.50. The method of Guggenheim could thus not be used for calculating the reaction

Table 4. Pseudo first order rate constants, "second order rate constants", and "second order rate constants" at ionic strength = 0 for several carboxylic acid esters of thiocholine,  $\alpha$ -methylthiocholine and  $\beta$ -methylthiocholine, determined at pH 9-10,  $t = 35^\circ\text{C}$ .

| 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> |      |      |       |
|---|--|---|------|-------|---|------|------|---|------|------|-------|
|   |  | 9.00  | 9.50 | 10.00 | 9.00  | 9.50 | 9.75 | 10.00   | 9.50 | 9.75 | 10.00 |
|   | pH   |   |      |       |   |      |      |   |      |      |       |
| CH <sub>3</sub> -   | $k_1 \text{ min}^{-1} \times 10^3$                           | 3.05  | 9.55 | 27.9  | 2.55  | 6.77 | 14.7 | 22.1  | 9.17 | 15.7 | 24.9  |
|   | " $k_2$ " $l \times \text{mole}^{-1} \times \text{min}^{-1}$ | 146   | 145  | 134   | 122   | 103  | 125  | 105   | 139  | 134  | 119   |
|   | " $k_3$ " $l \times \text{mole}^{-1} \times \text{min}^{-1}$ | 200   | 198  | 186   | 168   | 142  | 173  | 145   | 191  | 185  | 164   |
| CH <sub>3</sub> -CH <sub>2</sub> -  | $k_1 \text{ min}^{-1} \times 10^3$                           | 2.92  | 8.97 | 26.7  | 1.20  | 5.43 | 10.8 | 20.7  | 8.54 | 14.7 | 23.7  |
|   | " $k_2$ " $l \times \text{mole}^{-1} \times \text{min}^{-1}$ | 140   | 136  | 128   | 56.9  | 80.2 | 92.3 | 98.9  | 129  | 125  | 114   |
|   | " $k_3$ " $l \times \text{mole}^{-1} \times \text{min}^{-1}$ | 195   | 186  | 178   | 78.6  | 112  | 126  | 138   | 178  | 173  | 157   |
| CH <sub>3</sub> -CH <sub>2</sub> -CH <sub>2</sub> -                                   | $k_1 \text{ min}^{-1} \times 10^3$                           | 1.69  | 4.84 | 14.7  | 0.802   | 3.77 | 5.73 | 11.2  | 4.74 | 8.31 | 13.8  |
|   | " $k_2$ " $l \times \text{mole}^{-1} \times \text{min}^{-1}$ | 80.9  | 73.3 | 70.3  | 38.4  | 57.0 | 48.8 | 53.5  | 71.8 | 69.8 | 66.1  |
|   | " $k_3$ " $l \times \text{mole}^{-1} \times \text{min}^{-1}$ | 112   | 102  | 97.7  | 53.0  | 78.6 | 67.4 | 73.8  | 99.1 | 96.3 | 91.3  |
|    | $k_1 \text{ min}^{-1} \times 10^3$                           | 1.38  | 4.34 | 12.7  |   |      |      | 8.50  |      |      |       |
|   | " $k_2$ " $l \times \text{mole}^{-1} \times \text{min}^{-1}$ | 66.1  | 65.8 | 60.8  |   |      |      | 40.6  |      |      |       |
|   | " $k_3$ " $l \times \text{mole}^{-1} \times \text{min}^{-1}$ | 91.2  | 91.2 | 83.2  |   |      |      | 56.0  |      |      |       |
| CH <sub>3</sub> -CH <sub>2</sub> -CH <sub>2</sub> -CH <sub>2</sub> -                  | $k_1 \text{ min}^{-1} \times 10^3$                           | 1.67  | 4.95 | 14.7  | 0.854   | 2.32 | 4.98 | 10.9  | 5.08 | 8.16 | 12.8  |
|   | " $k_2$ " $l \times \text{mole}^{-1} \times \text{min}^{-1}$ | 80.0  | 75.0 | 70.3  | 40.8  | 35.2 | 42.4 | 52.1  | 76.9 | 69.5 | 61.2  |
|   | " $k_3$ " $l \times \text{mole}^{-1} \times \text{min}^{-1}$ | 110   | 105  | 97.7  | 56.2  | 48.4 | 58.5 | 71.9  | 106  | 96.0 | 84.4  |
|  | $k_1 \text{ min}^{-1} \times 10^3$                           | 1.44  | 4.17 | 13.3  | 0.640   | 2.06 | 4.67 | 7.78  | 4.00 | 7.26 | 11.6  |
|   | " $k_2$ " $l \times \text{mole}^{-1} \times \text{min}^{-1}$ | 68.9  | 63.5 | 63.7  | 30.5  | 31.1 | 39.7 | 37.2  | 60.5 | 61.8 | 55.6  |
|   | " $k_3$ " $l \times \text{mole}^{-1} \times \text{min}^{-1}$ | 95.5  | 87.1 | 87.1  | 42.1  | 42.9 | 54.9 | 51.3  | 83.5 | 85.2 | 76.6  |

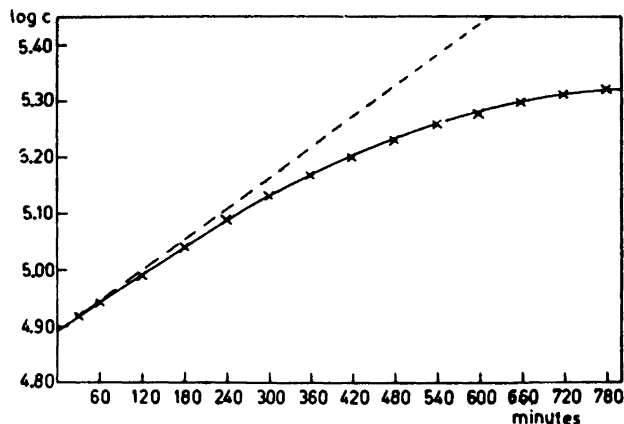


Fig. 2. Hydrolysis at pH 9.00. Logarithm of ester concentration for acetyl- $\beta$ -methylthiocholine, calculated from consumed amount of sodium hydroxide, plotted against time. Dotted line shows, how ester concentration should decrease, if the reaction was pseudo first order.  $t = 35^\circ\text{C}$ .

rates of these compounds at pH 9.00. The other values are shown in Table 4. The observed deviation from pseudo first order kinetics may depend upon another type of hydrolysis or upon secondary reactions, disturbing in the chosen method of analysis. To see if the hydrolysis of  $\beta$ -methylthiocholine esters at pH 9.00 proceeded as a first order reaction with respect to ester concentration,

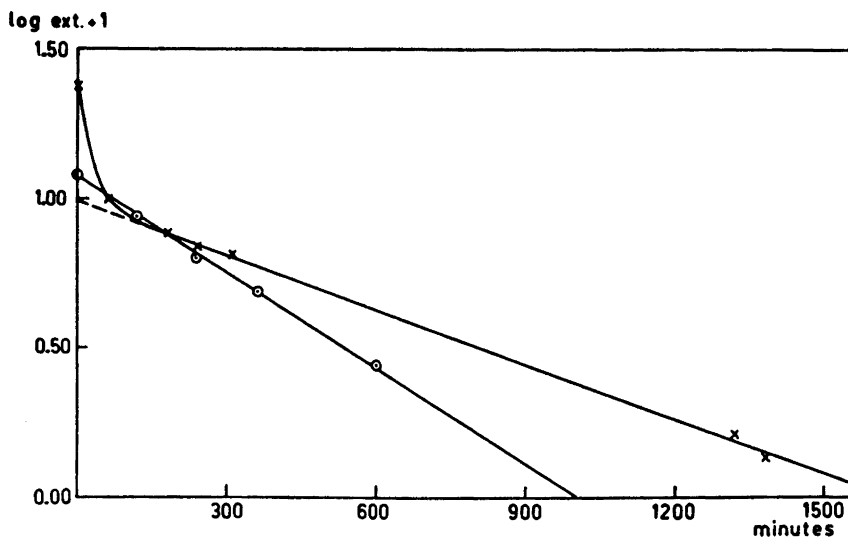


Fig. 3. Hydrolysis at pH 9.00. Logarithm of ester concentration for acetyl- $\beta$ -methylthiocholine (O) and butyryl- $\beta$ -methylthiocholine (x), expressed as logarithm of optical density of iron-hydroxamate complex, plotted against time.  $t = 35^\circ\text{C}$ .



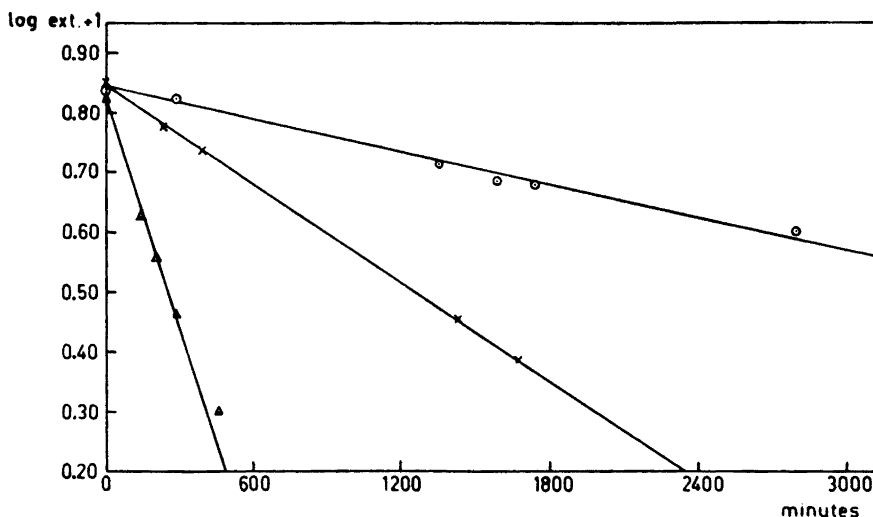
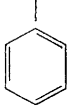


Fig. 4. Hydrolysis at pH 6 (○), 7 (×) and 8 (△). Logarithm of ester concentration of acetyl- $\alpha$ -methylthiocholine, expressed as logarithm of optical density of iron-hydroxamate complex, plotted against time.  $t = 55^\circ\text{C}$ .

the automatic recording titrator was used to keep the pH constant and the amount of unhydrolysed ester was measured as iron-hydroxamate complex. The initial concentration of the ester had to be higher than in earlier experiments because of the unsensitivity of the colorimetric method. As Fig. 3 shows, the pseudo first order rate constant for acetyl- $\beta$ -methylthiocholine was in this case  $0.00248 \text{ min}^{-1}$ , which would be the expected value, if the hydrolysis was dependent only upon hydroxyl ion activity. The hydrolysis seems to be first order with respect to ester concentration. The pseudo first order rate constant for butyryl- $\beta$ -methylthiocholine was, by the same method, found to be  $0.00137 \text{ min}^{-1}$ . The bend at the beginning of the curve may indicate that the sample, which was slightly hygroscopic, was not pure. The same type of curve was obtained with valeryl- $\beta$ -methylthiocholine.

*Hydrolysis of the thioesters at lower pH.* The rate of hydrolysis of thiocholine esters and their analogous was also determined in phosphate buffers at pH 6.0, 7.0 and 8.0 at  $55^\circ\text{C}$ . These buffer solutions could not keep the pH completely constant during hydrolysis. Owing to the acid character of the hydrolysis products a decrease of 0.10–0.15 pH units was observed. It is seen in Fig. 4 that the hydrolysis was first order with respect to ester concentration. First order rate constants, "second order rate constants", calculated according to eqn. (3), and " $k_2^0$ " calculated according to eqn. (4), are given in Table 5. The ionic strength is calculated from logarithm diagrams and the values were  $\sim 0.12$  at pH 6,  $\sim 0.20$  at 7 and  $\sim 0.31$  at pH 8. The value used for  $pK_w$  was 13.13. It is seen that proportionality to hydroxyl ion activity is not obtained:  $k_2^0$  increases as pH decreases.

Table 5. Pseudo first order rate constants, "second order rate constants" and "second order rate constants" at ionic strength = 0 for several carboxylic acid esters of thiocholine,  $\alpha$ -methylthiocholine and  $\beta$ -methylthiocholine determined at pH 6-8,  $t = 55^\circ\text{C}$ .

| 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-N <sup>+</sup> (CH <sub>3</sub> ) <sub>3</sub><br> <br>CH <sub>3</sub> |  |  | -CH-CH <sub>2</sub> -N <sup>+</sup> (CH <sub>3</sub> ) <sub>3</sub><br> <br>CH <sub>3</sub> |  |  |  |       |      |      |       |     |     |
|---|----------------|--|--|---|--|------|---|--|--|---|--|--|--|-------|------|------|-------|-----|-----|
|   | pH             | k <sub>1</sub> min <sup>-1</sup> × 10 <sup>4</sup> | "k <sub>2</sub> " l × mole <sup>-1</sup> × min <sup>-1</sup> | "k <sub>2</sub> " l × mole <sup>-1</sup> × min <sup>-1</sup>                      | "k <sub>2</sub> " l × mole <sup>-1</sup> × min <sup>-1</sup> | pH   | k <sub>1</sub> min <sup>-1</sup> × 10 <sup>4</sup>  | "k <sub>2</sub> " l × mole <sup>-1</sup> × min <sup>-1</sup> | "k <sub>2</sub> " l × mole <sup>-1</sup> × min <sup>-1</sup> | pH  | k <sub>1</sub> min <sup>-1</sup> × 10 <sup>4</sup> | "k <sub>2</sub> " l × mole <sup>-1</sup> × min <sup>-1</sup> | "k <sub>2</sub> " l × mole <sup>-1</sup> × min <sup>-1</sup> |       |      |      |       |     |     |
| CH <sub>3</sub> -   | 5.98           | 2.86   | 4 170  | 5 750   | 2 750  | 7.90 | 6.95  | 12.2   | 1 870  | 2 750   | 5.98   | 5.92   | 1.89   | 3 120 | 6.86 | 8.69 | 1 640 | 540 | 830 |
|   |                |  |  |   |  |      |   |  |  |   |  |  |  |       |      |      |       |     |     |
|   |                |  |  |   |  |      |   |  |  |   |  |  |  |       |      |      |       |     |     |
| CH <sub>3</sub> -CH <sub>2</sub> -  | 5.99           | 5.99   | 6.96   | 8.00  | 8.00   | 7.93 | 6.92  | 9.43   | 36.6   | 20.4  | 7.93   |  |  |       |      |      |       |     |     |
|   |                |  |  |   |  |      |   |  |  |   |  |  |  |       |      |      |       |     |     |
|   |                |  |  |   |  |      |   |  |  |   |  |  |  |       |      |      |       |     |     |
| CH <sub>3</sub> -CH <sub>2</sub> -CH <sub>2</sub> -                                   | 5.99           | 5.99   | 6.92   | 8.00  | 8.00   | 7.89 | 6.92  | 9.43   | 36.6   | 20.4  | 7.89   |  |  |       |      |      |       |     |     |
|   |                |  |  |   |  |      |   |  |  |   |  |  |  |       |      |      |       |     |     |
|   |                |  |  |   |  |      |   |  |  |   |  |  |  |       |      |      |       |     |     |
| CH <sub>3</sub> -CH <sub>2</sub> -CH <sub>2</sub> -CH <sub>2</sub> -                  | 5.95           | 5.95   | 6.98   | 8.00  | 8.00   | 7.93 | 6.98  | 9.43   | 36.6   | 20.4  | 7.93   |  |  |       |      |      |       |     |     |
|   |                |  |  |   |  |      |   |  |  |   |  |  |  |       |      |      |       |     |     |
|   |                |  |  |   |  |      |   |  |  |   |  |  |  |       |      |      |       |     |     |
|  | 5.97           | 5.97   | 6.96   | 8.00  | 8.00   | 7.93 | 6.96  | 9.43   | 36.6   | 20.4  | 7.93   |  |  |       |      |      |       |     |     |
|   |                |  |  |   |  |      |   |  |  |   |  |  |  |       |      |      |       |     |     |
|   |                |  |  |   |  |      |   |  |  |   |  |  |  |       |      |      |       |     |     |

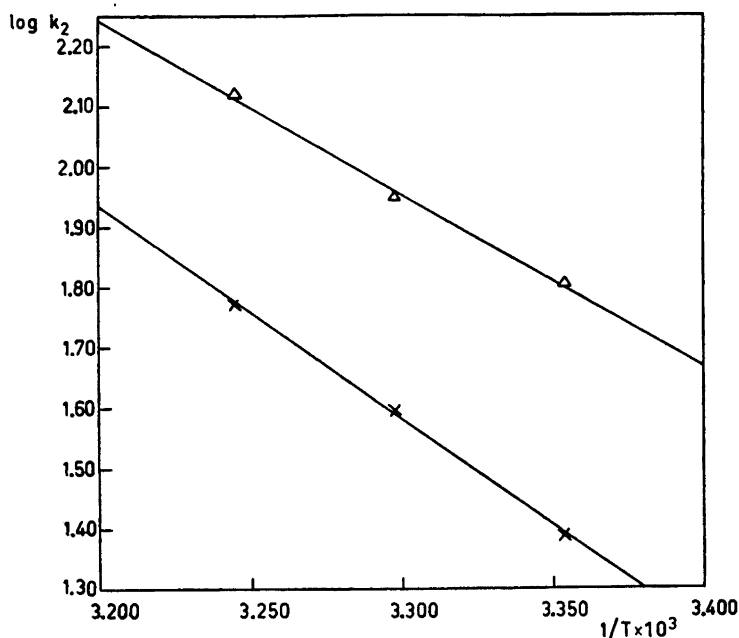


Fig. 5. Temperature dependence of hydrolysis. Second order rate constant in  $l \times \text{mole}^{-1} \times \text{min}^{-1}$  plotted against  $1/T$ . Line calculated by method of least squares.  $\Delta$  = acetylthiocholine,  $\times$  = acetyl- $\beta$ -methylcholine. pH = 10.00.

### Temperature dependence

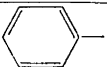
The temperature dependence of the rate of hydrolysis for choline and thiocholine esters and methyl substituted analogues of both was determined at pH 10.00 at 25°C, 30°C and 35°C. The logarithms of the second order rate constants, calculated as described earlier, were plotted against  $1/T$  as shown in Fig. 5. The line

$$\log k_2 = A_{\text{OH}^-} + B_{\text{OH}^-}/T \quad (3)$$

Table 6. Arrhenius' constants and activation energies for acetylcholine, acetyl- $\alpha$ -methylcholine and acetyl- $\beta$ -methylcholine. pH = 10.00.

| 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> — $\overset{\text{CH}_3}{\text{CH}}$ —N <sup>+</sup> (CH <sub>3</sub> ) <sub>3</sub> |                             |        | — $\overset{\text{CH}_3}{\text{CH}}$ —CH <sub>2</sub> —N <sup>+</sup> (CH <sub>3</sub> ) <sub>3</sub> |                             |        |
|-------------------|---|-----------------------------|--------|---|-----------------------------|--------|---|-----------------------------|--------|
|                   | A <sub>OH<sup>-</sup></sub>   | B <sub>OH<sup>-</sup></sub> | E kcal | A <sub>OH<sup>-</sup></sub>   | B <sub>OH<sup>-</sup></sub> | E kcal | A <sub>OH<sup>-</sup></sub>   | B <sub>OH<sup>-</sup></sub> | E kcal |
| CH <sub>3</sub> - | 11.4 ± 0.8  | 2780 ± 260                  | 12.7   | 11.2 ± 0.6  | 2730 ± 180                  | 12.5   | 13.3 ± 0.2  | 3550 ± 150                  | 16.2   |

Table 7. Arrhenius' constants and activation energies for several carboxylic acid esters of thiocholine,  $\alpha$ -methylthiocholine and  $\beta$ -methylthiocholine. The error is calculated by regression analysis. No error given means only 3 determinations. pH = 10.00.

| 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> — $\underset{\text{CH}_3}{\text{CH}}$ —N <sup>+</sup> (CH <sub>3</sub> ) <sub>3</sub> |                              |        | — $\underset{\text{CH}_3}{\text{CH}}$ —CH <sub>2</sub> —N <sup>+</sup> (CH <sub>3</sub> ) <sub>3</sub> |                              |        |
|---|---|------------------------------|--------|--|------------------------------|--------|--|------------------------------|--------|
|   | A <sub>OH</sub> <sup>-</sup>  | B <sub>OH</sub> <sup>-</sup> | E kcal | A <sub>OH</sub> <sup>-</sup>   | B <sub>OH</sub> <sup>-</sup> | E kcal | A <sub>OH</sub> <sup>-</sup>   | B <sub>OH</sub> <sup>-</sup> | E kcal |
| CH <sub>3</sub> —   | 11.5 ± 0.6  | 2760 ± 350                   | 12.6   | 11.0   | 2 760                        | 12.6   | 10.7   | 2 660                        | 12.1   |
| CH <sub>3</sub> —CH <sub>2</sub> —  | 11.1 ± 1.5  | 2780 ± 450                   | 12.7   | 10.8 ± 0.6   | 2720 ± 240                   | 12.4   | 10.7 ± 0.5   | 2670 ± 140                   | 12.1   |
| CH <sub>3</sub> —CH <sub>2</sub> —CH <sub>2</sub> —                               | 11.2 ± 0.6  | 2870 ± 170                   | 13.1   | 11.3 ± 2.3   | 2830 ± 650                   | 12.9   | 10.8 ± 0.9   | 2729 ± 280                   | 12.4   |
| (CH <sub>3</sub> ) <sub>2</sub> CH—   | 12.7 ± 0.7  | 3360 ± 220                   | 15.4   |  |                              |        |  |                              |        |
| CH <sub>3</sub> —CH <sub>2</sub> —CH <sub>2</sub> —CH <sub>2</sub> —              | 11.6 ± 0.9  | 3020 ± 260                   | 13.8   | 11.2   | 2930                         | 13.4   | 10.9 ± 0.9   | 2810 ± 290                   | 12.9   |
|  | 13.0 ± 0.8  | 3450 ± 230                   | 15.9   | 11.6   | 3 070                        | 14.0   | 11.3 ± 0.5   | 2960 ± 160                   | 13.5   |

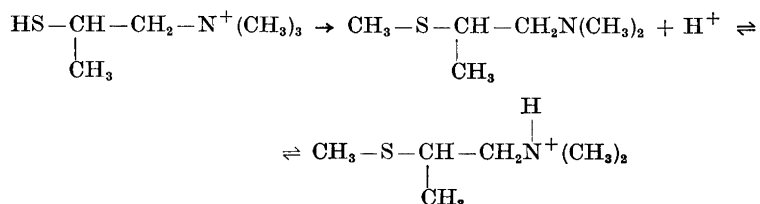
representing the Arrhenius' equation, was calculated by means of the method of least squares. The activation energy was also calculated. The logarithm for the collision factor, PZ, is equal to A<sub>OH</sub><sup>-</sup>. The following values for pK<sub>w</sub> were used: pK<sub>w</sub> 25°C = 14.00, pK<sub>w</sub> 30°C = 13.83 and pK<sub>w</sub> 35°C = 13.68. The constants in the Arrhenius' equation and the activation energies are given in Tables 6 and 7.

#### DISCUSSION

Only a slight difference in the rate of hydrolysis of the thiocholine esters and the two methylthiocholine esters was found at pH 10.00 as long as the acid residue of the esters was the same. The unbranched thiocholine esters were found to hydrolyse fastest, followed by  $\beta$ -methyl substituted esters. Slowest hydrolysis showed the  $\alpha$ -methyl substituted esters. Even activation energies and PZ factors were found to be almost equal for the three types of esters. This is obviously different from the corresponding choline esters. The hydrolysis of unsubstituted and  $\alpha$ -methyl substituted choline esters was found to proceed faster than that of  $\beta$ -methylcholine esters. This is also indicated by the activation energies of the choline esters, which were found to be about 12.6 kcal/mole for unsubstituted and  $\alpha$ -methyl substituted esters, but 16.2 kcal/mole for  $\beta$ -methylsubstituted esters. The reason for this difference may be found in the steric effect of the  $\beta$ -methyl group. The radius of the oxygen atom, according to Pauling 0.66 Å, and the resulting length of the

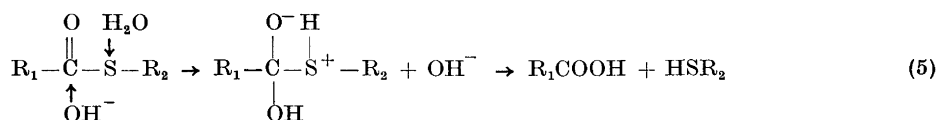
C—O—C bridge (2.20 Å) seems to make it possible for the  $\beta$ -methyl group to provide a screen against an approaching hydroxyl ion, but the bigger sulphur atom, according to the same author 1.04 Å, and giving rise to a greater length of the C—S—C bridge (2.58 Å) leaves more space for the attack of the approaching hydroxyl ion at the carbonyl carbon and seems to counteract the effect of the  $\beta$ -methyl group. The observed influence of the substituents of the acid residue upon the rate of hydrolysis is greater than the effect of the substituents of the thiol residue. The reaction rate decreases with the length of the carbon chain, methyl and ethyl showing nearly the same rate, and then the reaction rate falling to about 50 % of the value found for methyl, when the substituents are propyl or butyl; phenyl and *isopropyl* substituted esters showing a still smaller rate of reaction. These observations may be explained by the combined steric (*e.g.*, *isopropyl*) and inductive (*e.g.*, phenyl) effects of the substituents upon the attacked carbonyl carbon.

The observed deviations at lower pH, especially with the  $\beta$ -methyl-thiocholine esters, from pseudo first order kinetics in experiments with the automatic recording titrator seem to depend upon secondary reactions, probably by the products of hydrolysis. This is indicated by the unknown spots in the paper chromatograms and ionophoretograms of the hydrolysates. A possible reaction would be

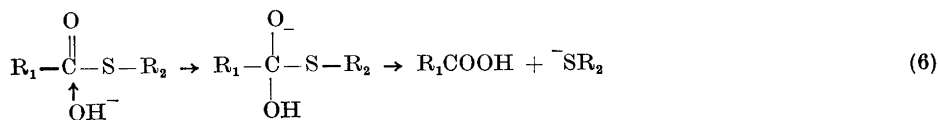


The  $pK_a$  of the latter compound is probably more than 9, which would offer an explanation of the smaller amount of alkali used by  $\beta$ -methyl-thiocholine esters for complete hydrolysis at pH 9.

The found activation energies for choline esters and thiocholine esters are near the activation energies found by other workers<sup>1,3</sup> for other thioesters. Together with the found pseudo first order hydrolysis and the found products of hydrolysis this indicates, that the mechanism of the alkaline hydrolysis of the studied esters may be described by the Lowry mechanism for ordinary ester hydrolysis, *viz.*



However, the bond S— — —HOH should be weaker<sup>21</sup> than O— — —HOH, so perhaps solvation is less influential for thioesters. In this case the mechanism of alkaline hydrolysis could perhaps be described by



Such a mechanism would make it still easier to understand the nonexistent steric effect of the  $\beta$ -methyl group in the thiol.

The increasing value for " $k_2^0$ " observed in rate reactions in phosphate buffer solutions may indicate catalysis by phosphate ions. In earlier studies on choline esters, performed with the same methods and in the same medium by Larsson<sup>22</sup>, this effect was not observed. However, there is a factor of about two between the rate constants for choline esters found by Larsson and those found now with the automatic titrator, which supports the possibility of a catalysis by phosphate ions. In that case the catalytic activity comes from the ions  $\text{H}_2\text{PO}_4^-$  and  $\text{HPO}_4^{2-}$  and their effect seems to be greater upon thioesters than upon oxygen esters. There is also the possibility of explaining the increasing " $k_2^0$ " values by assuming two competitive reactions, each with a reaction rate proportional to ester concentration. These reactions could for instance be hydrolysis according to eqns. (5) and (6) described earlier, where reaction (6) would prevail at higher pH-values.

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