[CONTRIBUTION FROM THE INSTITUTE FOR ENZYME RESEARCH AND THE DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF WISCONSIN]

Properties of Thiolesters: Kinetics of Hydrolysis in Dilute Aqueous Media[1]

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Ultraviolet absorption spectra have been measured for a number of thiolacetates and mercaptans in methyl alcohol, aqueous acid and aqueous alkaline media. The rates of reaction in very dilute aqueous media have been measured by means of a spectrophotometric method. Three general cases have been studied: acid hydrolysis, alkaline hydrolysis and reaction with hydroxylamine. The rate constants and values for energy of activation and log *PZ* have been determined for each case. The Lowry mechanism for ester hydrolysis has been extended to include the three types of reaction.

Biochemists recently have become aware of the important role of thiolesters in many biological systems. Chibnall[4] has suggested that thiolester linkages may contribute to the structure of proteins; Racker[5] has shown that the glyoxalase enzymes act by the intermediate formation of a thiolester in which the coenzyme glutathione participates. The brilliant work of Lynen[6] on Coenzyme A demonstrated that the combination of this coenzyme with "activated" acyl groups is in fact a thiolester. The importance of this coenzyme in intermediary metabolism is widely recognized.[7]

An understanding of the physical and chemical properties of the thiolesters should aid in understanding biochemical mechanisms involving compounds of this type. Although considerable information is available[8] much of it is inapplicable to biological systems. Schaefgen[9] and Rylander and Tarbell[10] have studied the hydrolysis of thiolesters in aqueous acetone media and have proposed mechanisms for the hydrolysis. Sjöberg[11] measured the absorption spectra of a number of sulfur compounds and showed that thiolesters have an absorption maximum at about 230 mμ. Racker[5] measured the enzymatic formation of a thiolester from methylglyoxal and glutathione by the absorption at 240 mμ.

The present work deals with the kinetics of hydrolysis of several thiolacetates in *very dilute aqueous* media. Three general cases were studied: (a) hydrolysis in acid, (b) hydrolysis in alkali and (c) the reaction with hydroxylamine (where the hydroxamic acid is formed). Rates of hydrolysis were determined spectrophotometrically which necessitated measurements of the absorption maxima and molar extinction coefficients.

The absorption spectra of a typical thiolester and a mercaptan under various conditions are shown in Fig. 1. A small increase in the maximum for *n*-butyl thiolacetate and a slight shift to longer wave lengths are observed in changing the solvent from methyl alcohol to water (distilled H_2O or 0.01 *N* HCl). In alkali, Na_2S and *n*-butyl mercaptide ion display absorption curves similar to that of the thiolester; however, in acid, H_2S (not shown in Fig. 1), and *n*-butyl mercaptan essentially lose their ultraviolet absorption. Thus it is possible to measure the concentration of thiolester and to follow the course of reaction in very dilute aqueous solutions, avoiding the use of solvents which other workers found necessary for reasons of solubility.[9,10]

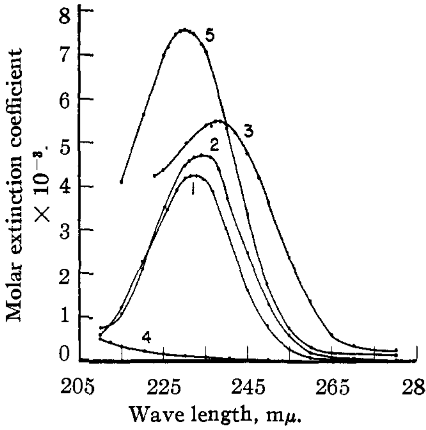
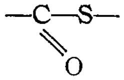


Fig. 1.—Absorption spectra of *n*-butyl thiolacetate, butyl mercaptan and sodium sulfide: 1, *n*-butyl thiolacetate in MeOH; 2, *n*-butyl thiolacetate in H_2O; 3, *n*-butyl mercaptan in 1 *N* NaOH; 4, *n*-butyl mercaptan in 0.01 *N* HCl; 5, sodium sulfide (Mallinckrodt A.R.) in 0.01 *N* NaOH.

Table I summarizes the data on the ultraviolet absorption spectra. The molar extinction coefficient of thiolacetates in methyl alcohol is about 4.4 × 10^3, compared to about 0.22 × 10^3 for the mercaptan in methyl alcohol.

Figure 2 shows the infrared absorption spectra of ethyl thiolacetate and of *n*-butyl thiolacetate. Tentatively, the peak at about 9 microns is assigned to the C–S stretching in the



$-\underset{\substack{\| \\ \mathrm{O}}}{\mathrm{C}}-\mathrm{S}-$ group.[12]

Acid Hydrolysis.—Schaefgen[9] and Rylander and Tarbell[10] found the acid-catalyzed hydrolysis in

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[2] Fellow of the National Heart Institute.

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[4] A. C. Chibnall, *Proc. Roy. Soc.* (*London*), **B131**, 136 (1942).

[5] E. Racker, *J. Biol. Chem.*, **190**, 685 (1951).

[6] (a) F. Lynen and E. Reichert, *Angew. Chem.*, **63**, 47, 474 (1951); (b) F. Lynen, E. Reichert and L. Rueff, *Ann.*, **574**, 1 (1951).

[7] (a) M. Soodak and F. Lipmann, *J. Biol. Chem.*, **175**, 999 (1948); (b) S. Korkes, J. R. Stern, I. C. Gunsalus and S. Ochoa, *Nature*, **166**, 439 (1950); (c) E. R. Stadtman, G. D. Novelli and F. Lipmann, *J. Biol. Chem.*, **191**, 365 (1951); (d) F. Lipmann, M. E. Jones, S. Black and R. M. Flynn, THIS JOURNAL, **74**, 2384 (1952); (e) D. R. Sanadi and J. W. Littlefield, *J. Biol. Chem.*, **193**, 683 (1951); (f) G. Drysdale and H. A. Lardy, *ibid.*, in press.

[8] D. S. Tarbell and D. P. Harnish, *Chem. Revs.*, **49**, 1 (1951).

[9] J. R. Schaefgen, THIS JOURNAL, **70**, 1308 (1948).

[10] P. N. Rylander and D. S. Tarbell, *ibid.*, **72**, 3021 (1950).

[11] B. Sjöberg, *Z. physik. Chem.*, **52B**, 209 (1942).

[12] We are indebted to Mr. Donald Johnson of the Instrumental Analysis Laboratory of the Department of Chemistry, University of Wisconsin, for the infrared absorption measurements and interpretation.

TABLE I

MOLAR EXTINCTION COEFFICIENTS OF THIOLACETATES AND MERCAPTANS IN METHYL ALCOHOL

Compound	B.p. (mm.)[a] or m.p.,[a] °C.	$\epsilon \times 10^{-3}$ at[b] 231 mμ
n-Butyl thiolacetate	159–160 (738)	4.3[c]
i-Butyl thiolacetate	149–150 (737)	4.4
t-Butyl thiolacetate	44–45 (28)	4.3
n-Amyl thiolacetate	70–71 (14)	4.5
i-Amyl thiolacetate	174–175 (737)	4.5
n-Hexyl thiolacetate	202–203 (746)	4.5
n-Octyl thiolacetate	114–115 (13)	4.4
n-Nonyl thiolacetate	128–129 (13)	4.5
n-Decyl thiolacetate	140–141 (13)	4.4
Acetylthioglycolic acid	149–150 (13)	3.4[d]
Acetylmercaptopropionic acid (s)	52–54	4.2
Acetylthiomalic acid (s)	125–126	3.7
NS-Diacetylcysteine (s)	111–112	3.9
n-Butyl mercaptan	94–95 (740)	0.13
Thioglycolic acid	104 (15)	.22[e]
N-Acetylcysteine (s)	107–108	.20

[a] Uncorrected. The melting and boiling points agree reasonably well with literature values. The solid compounds are indicated by (s). [b] 231 mμ is the absorption maximum in methyl alcohol for the thiolacetates listed. [c] ϵ_{233} mμ (max) 4.8×10^{-3} in 0.01 *N* aq HCl. [d] ϵ_{230} mμ (max) 4.1×10^3 in 0.01 *N* aq. HCl. [e] ϵ_{231} mμ 0.22×10^3 in 0.01 *N* aq. HCl.

aqueous acetone solution to be pseudo first order with respect to thiolester concentration. Figure 3 illustrates that the hydrolysis of isobutyl thiolacetate is first order with respect to thiolester and hydrogen ion concentration. At the relatively high concentration of hydrogen ion compared to thiolester, and because the hydrogen ion is regenerated during the reaction (a true catalysis), the over-all kinetics should be first order. This is confirmed by the linearity of first-order plots to at least 80% hydrolysis.

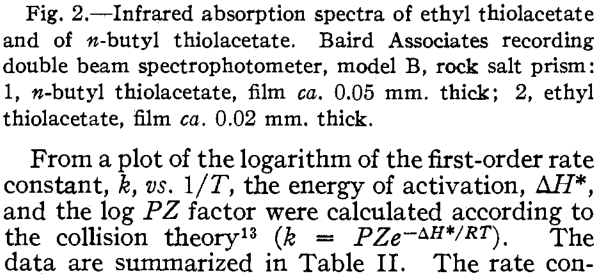

Fig. 2.—Infrared absorption spectra of ethyl thiolacetate and of *n*-butyl thiolacetate. Baird Associates recording double beam spectrophotometer, model B, rock salt prism: 1, *n*-butyl thiolacetate, film *ca.* 0.05 mm. thick; 2, ethyl thiolacetate, film *ca.* 0.02 mm. thick.

From a plot of the logarithm of the first-order rate constant, *k*, *vs.* $1/T$, the energy of activation, ΔH^*, and the log *PZ* factor were calculated according to the collision theory[13] ($k = PZe^{-\Delta H^*/RT}$). The data are summarized in Table II. The rate constants for all the thiolesters studied are of the same order of magnitude and the values for energy of activation and log *PZ* are essentially constant. However, Rylander and Tarbell[10] found that for the acid hydrolysis in aqueous acetone solution there was a progressive increase in the energies of activation of methyl, isopropyl, isobutyl and *t*-butyl thiolacetates. In addition, an increase in acetone concentration resulted in lower velocity constants and higher energies of activation. This effect of acetone on the reaction velocity may result from an alteration in dielectric constant producing a change in ΔH^* or a direct interaction of the acetone with one of the reacting species, *e.g.*, H_3O^+.

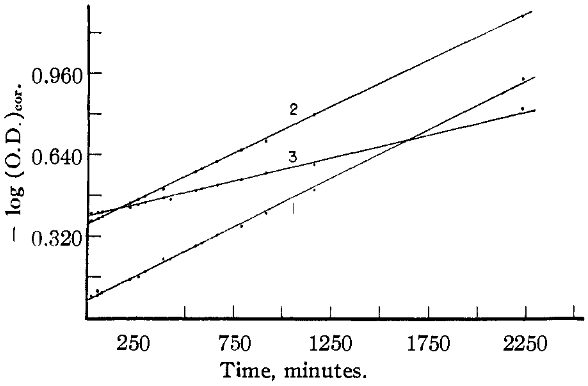

Fig. 3.—Acid-catalyzed hydrolysis of isobutyl thiolacetate at 30°. Variation of initial concentration of reactants: 1, 2.02×10^{-4} *M* thiolacetate, 1.00 *N* HCl, $k = 8.43 \times 10^{-4}$, $k/[\text{HCl}] = 8.43 \times 10^{-4}$; 2, 1.01×10^{-4} *M* thiolacetate, 1.00 *N* HCl, $k = 8.35 \times 10^{-4}$, $k/[\text{HCl}] = 8.35 \times 10^{-4}$; 3, 1.01×10^{-4} *M* thiolacetate, 0.500 *N* HCl, $k = 4.06 \times 10^{-4}$, $k/[\text{HCl}] = 8.12 \times 10^{-4}$.

TABLE II

RATE CONSTANTS AND ACTIVATION ENERGIES FOR THE ACID-CATALYZED HYDROLYSIS OF THIOLACETATES IN AQUEOUS SOLUTIONS

Thiolester	$k_{25°} \times 10^4$[a] $[\text{HCl}]_{\text{initial}}$	$k_{30°} \times 10^4$[a] $[\text{HCl}]_{\text{initial}}$	$k_{37.7°} \times 10^4$[a] $[\text{HCl}]_{\text{initial}}$	ΔH^*[b]	log *PZ*[c]
n-Butyl thiolacetate	6.24	9.95	20.2	17.9	10.0
i-Butyl thiolacetate	5.67	8.39	18.3	17.9	9.9
t-Butyl thiolacetate	5.21	8.05	15.7	16.8	9.0
n-Amyl thiolacetate	6.27	10.7	20.5	18.1	10.1
i-Amyl thiolacetate	6.72	10.7	20.4	16.8	9.1
n-Hexyl thiolacetate	6.58	10.1	19.7	16.8	9.1
Acetylthioglycolic acid	4.18	6.49	13.6	18.1	9.9
Acetylmercapto-propionic acid	4.36	7.44	14.0	17.8	9.7
Acetylthiomalic acid	3.31	5.39	10.8	18.0	9.7
NS-Diacetylcysteine	3.99	7.30	13.0	18.0	9.8

[a] *k* expressed in min.$^{-1}$; the $[H_2O]$ is neglected. [b] ΔH^* expressed in kcal./mole. [c] log *PZ* calculated from the values for $k/[\text{HCl}]$.

In this study, the relative constancy of ΔH^* and log *PZ* for 10 thiolacetates indicates a similar mechanism of hydrolysis for all the thiolacetates studied.

Alkaline Hydrolysis.—The rate of hydrolysis of thiolesters in alkaline solution is tremendously greater than in acid. The second-order velocity constants were determined graphically from a plot of log $(b(a - 2x)/a(b - x))$ *vs.* time.[14] Linearity

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(13) E. A. Moelwyn-Hughes, "The Kinetics of Reactions in Solution," Oxford University Press, Oxford, 1947, p. 138.

(14) The reaction follows the second order rate equation which corrects for an additional mole of hydroxyl ion neutralized by the liberated mercaptan

$$\frac{1}{a - 2b} \ln \frac{b}{a} \frac{(a - 2x)}{(b - x)} = kt$$

where $a = [\text{OH}^-]_{\text{initial}}$ and $b = [\text{thiolester}]_{\text{initial}}$; see Schaefgen.[9]

of these plots to 80% hydrolysis is confirmation of the postulated second-order mechanism. Figure 4 shows a plot for acetylthioglycolic acid at a number of different temperatures. Table III summarizes the data and shows that although there is about a 9-fold variation in the rate constant between the slowest reacting (acetylthiomalic acid) and the most rapidly reacting (NS-diacetylcysteine) compounds, the values for ΔH^* and log PZ are essentially constant. Previous workers[10] found that there was a difference of about 4 kcal./mole between the energies of activation for methyl thiolacetate and *t*-butyl thiolacetate reacting in aqueous acetone solution. The energies of activation increased and velocity constants decreased with increasing acetone concentration.

TABLE III

RATE CONSTANTS AND ENERGIES OF ACTIVATION FOR THE ALKALINE HYDROLYSIS OF THIOLESTERS IN AQUEOUS SOLUTION

Thiolester	$k_{25°}$[a]	$k_{30°}$[a]	$k_{37°}$[a]	ΔH^*[b]	log PZ[c]
n-Butyl thiolacetate	4.79	7.13	11.3	13.3	10.5
Acetylthioglycolic acid	2.97	3.63	7.56	14.2	10.9
Acetylthiomalic acid	0.884	1.33	2.27	14.4	10.5
NS-Diacetylcysteine	7.35	11.0	18.3	14.0	11.2

[a] k expressed in l./mole./min. [b] ΔH^* expressed in kcal./mole. [c] log PZ calculated from the second-order rate constants.

Because of the constancy of the energy of activation and entropy factor in our experiments, the mechanism of alkaline hydrolysis for the four thiolacetates having substituents of differing electronegativity would appear to be the same.

Reaction with Hydroxylamine.—An acid *p*H was chosen to follow the course of reaction in order to minimize the extremely rapid alkaline hydrolysis. Since the initial concentration of un-ionized hydroxylamine (0.01 *M*) was about 50 times greater than the concentration of the thiolacetate, the concentration of hydroxylamine can be assumed to be essentially constant during the initial course of the reaction.[15] Plots of negative logarithm of the optical density *vs.* time were linear, up to 60% hydrolysis, and thus the over-all reaction is pseudo first order.

The Lipmann and Tuttle[17] ferric chloride test for hydroxamic acid was used to follow the increasing formation of acethydroxamic acid at the same time that the decrease in thiolester was measured by the absorption at 231 mμ. Plots of the negative logarithm of the concentration of thiolester *vs.* time were linear and gave a rate constant of 0.0475 by the hydroxamic test and 0.0518 by the spectrophotometric method. The two constants agree within 8.3% and indicate that the reaction with hydroxylamine must yield acethydroxamic acid and mercaptan with no serious competing side reactions. Rate constants for the hydroxylamine reaction determined by the ultraviolet absorption method fitted the Arrhenius plots within an error of about 8%.

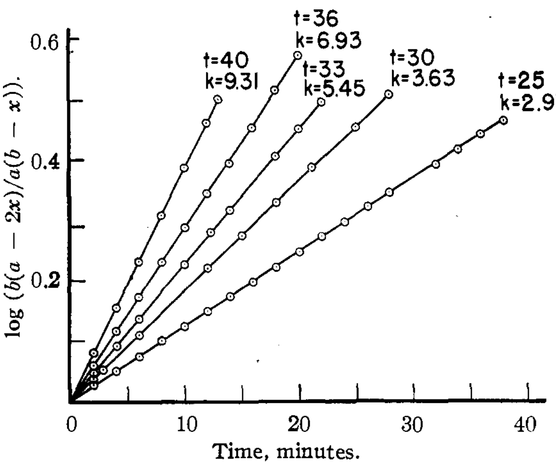

Fig. 4.—Alkaline hydrolysis of acetylthioglycolic acid at various temperatures: 0.01012 *N* NaOH, 3.0-3.3 × 10^{-4} *M* acetylthioglycolic acid, k expressed in l./ mole/min.

Reaction of esters and anhydrides with hydroxylamine is *p*H dependent.[18] More specifically, the rate of reaction depends upon the concentration of un-ionized hydroxylamine. In an experiment with 0.01 *M* hydroxylamine and 0.04 *M* hydroxylamine hydrochloride (*p*H 5.4), the slope of the line for the plot of the logarithm of the corrected optical density of acetyl thioglycolic acid *vs.* time was 0.0271 and the rate constant/[NH_2OH] was 6.2 at 20°. With 0.02 *M* hydroxylamine and 0.03 *M* hydroxylamine hydrochloride (*p*H 5.9) the slope of the line was 0.0521 and the rate constant/[NH_2OH] 6.0. The slope was nearly doubled and the rate constants agreed within experimental error. Thus the reaction is first order with respect to un-ionized hydroxylamine.

Table IV summarizes the data. In the reaction of thiolacetate with hydroxylamine there is greater variation of the rate constants and values for ΔH^* and log PZ than in acid-catalyzed or alkaline hydrolysis. There appears to be no correlation between the rate constants and the values of ΔH^* or log PZ. It is of interest that of all the thiolacetates studied, acetylthiomalic acid, which showed the slowest rates of hydrolysis in acid and alkali, reacted most rapidly with hydroxylamine.

TABLE IV

RATE CONSTANTS AND ACTIVATION ENERGIES FOR THE REACTION OF THIOLACETATES WITH HYDROXYLAMINE IN AQUEOUS SOLUTION

Thiolester	$k_{20°}$[a] [NH_2-OH]	$k_{25°}$[a] [NH_2-OH]	$k_{30°}$[a] [NH_2·OH]	ΔH^*[b]	log PZ[c]
n-Butyl thiolacetate	0.551	0.720	1.04	11.6	8.4
Acetylthioglycolic acid	6.11	7.96	10.2	8.8	7.4
Acetylthiomalic acid	13.3	18.2	22.6	10.4	8.9
NS-Diacetylcysteine	1.77	2.29	2.58	6.6	5.3

[a] k expressed in min.$^{-1}$; [b] ΔH^* expressed in kcal./mole; [c] log PZ calculated from values for k/[NH_2OH].

Comparative Kinetic Data for Acetylthioglycolic Acid.—The Arrhenius plots for the three different reactions of acetylthioglycolic acid are shown in

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[15] The *p*H (glass electrode) before and after hydrolysis was found to be constant at about *p*H 5.4 for the different runs and in good agreement with the *p*H of 5.38 calculated from the acid dissociation constant of 1.04 × 10^{-6} given by Hagisawa.[16]

[16] H. Hagisawa, *Bull. Inst. Phys. Chem. Research (Tokyo)*, **20**, 251 (1941).

[17] F. Lipmann and L. C. Tuttle, *J. Biol. Chem.*, **159**, 21 (1945).

[18] S. Hestrin, *ibid.*, **180**, 249 (1949).

Fig. 5. The hydroxylamine first-order rate constant is about 10,000 times greater than that of the acid hydrolysis first-order rate constant. The rate of hydrolysis of acetylthioglycolic acid with alkali is about 7,000 times greater than with an equivalent amount of acid. Reaction of acetylthioglycolic acid with hydroxylamine is about 2.7 times more rapid than the alkaline hydrolysis. There is correlation between the rate of reaction and the energy of activation but not with the log *PZ* factors. An explanation for this is offered later.

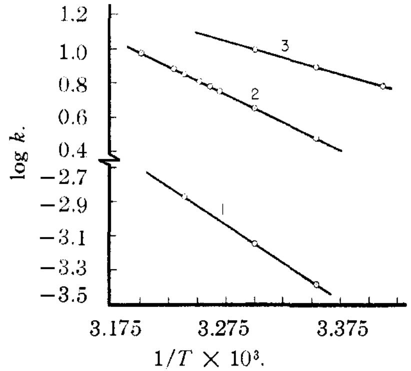
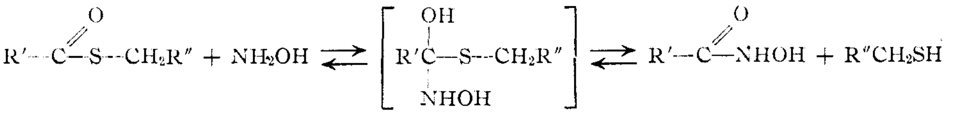


Fig. 5.—Arrhenius plots for reaction of acetyl thioglycolic acid: 1, acid hydrolysis; initial [HCl] = 1.00 *N*, initial [Ac-S-CH_2-COOH] = 1.6-2.0 × 10^{-4} *M*, ΔH^* = 18.1 kcal./mole, log *PZ* = 9.9; 2, alkaline hydrolysis; initial [OH^-] = 0.00980 *N*, initial [Ac-S-CH_2-COOH] = 2.8-3.3 × 10^{-4} *M*, ΔH^* = 14.2 kcal./mole, log *PZ* = 10.9; 3, reaction with hydroxylamine; initial [H_2NOH] = 0.010 *M*, initial [Ac-S-CH_2-COOH] = 1.6-2.1 × 10^{-4} *M*, ΔH^* = 8.8 kcal./mole, log *PZ* = 7.4.

Reaction with Other Compounds.—Thiolesters react readily with hydrazine, semicarbazide and other substituted hydrazines, presumably by a mechanism similar to the reaction with hydroxylamine. The inactivation of enzymes by these "carbonyl group reagents"[19] could conceivably be due to reaction with a thiolester group on the enzyme. 2,4-Dinitrophenol did not catalyze the hydrolysis of thiolesters at *p*H 7.4 (phosphate buffer).

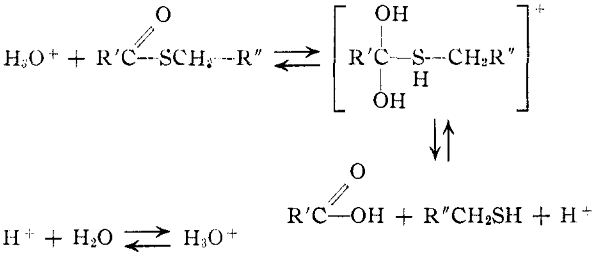
Mechanisms of Hydrolysis

Reaction with Hydroxylamine.—The observation that un-ionized hydroxylamine is probably the reactant leads to the plausible mechanism



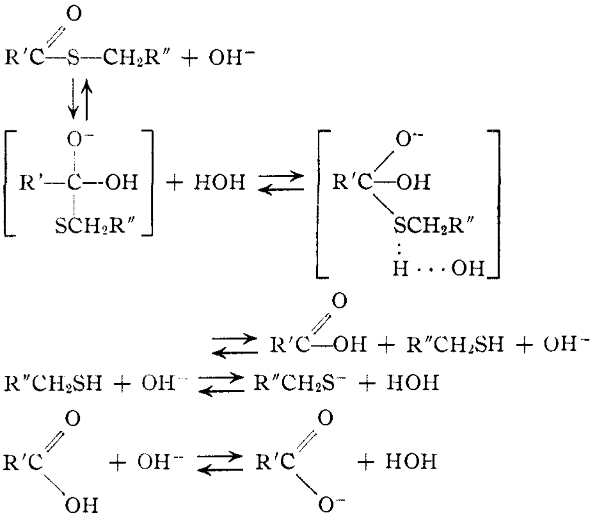
which is consistent with the experimental finding that the reaction is first order with respect to both hydroxylamine and thiolester and also consistent with a steady state analysis.

Hydrolysis in Acid and Alkali.—The generalized Lowry mechanisms[20] suitable for the O-esters may be aptly extended to include the thiolesters in aqueous media with the reservation that the mechanisms include only bimolecular and unimolecular steps. For acid hydrolysis



Assuming the first reaction is the rate-controlling step, the resultant steady state equation is found to be first order with respect to [H^+], [H_2O], and [thiolester] or third order over-all kinetics. This was confirmed experimentally by reducing the water concentration to the level equivalent to the thiolester (which could be done in the case of acetylthioglycolic acid).

Similarly for alkaline hydrolysis



where the rate-limiting step would be the initial attack by OH^- on the thiolester. This results in second order over-all kinetics as revealed by a steady state analysis and experimental verification which corrects for the fact that two moles of OH^- are utilized by the reaction, leading to the expression[9]

$$dx/dt = k(a - 2x)(b - x)$$ [9]

A comparison of the three proposed general mechanisms for NH_2OH, H_3O^+ and OH^- reveals that the rate-limiting step is an attack essentially on the carbonyl C atom, either by N (of NH_2OH) or O (of OH^- or H_3O^+). In very dilute aqueous media containing NH_2OH, all three general mechanisms of hydrolysis are assumed to be operating, but which mechanism predominates will of course depend upon experimental conditions. Thus under the conditions used here for NH_2OH, the reaction by H_3O^+ and OH^- becomes negligible and that by NH_2OH overwhelmingly predominates. The average ΔH^* values for the three cases are 9, 14 and 18 kcal./mole for NH_2OH, OH^- and H_3O^+,

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(19) O. Schales, A. M. Suthon, R. M. Rous, E. Lloyd and S. S. Schales, *Arch. Biochem.*, **19**, 119 (1948); O. Schales and B. R. Hill, *ibid.*, **22**, 366 (1949).

(20) T. M. Lowry, *J. Chem. Soc.*, 1381 (1925); 2554 (1927).

respectively. This is exemplified by Fig. 5 for acetylthiolglycolic acid. The rates are many orders of magnitude higher for the reaction with NH_2OH or OH^- than for H_3O^+ (reaction with NH_2OH being *ca.* 3 times faster than with OH^-). There is thus a good correlation between rates and ΔH^* values. The values for log *PZ* average about 8 for reaction with NH_2OH, about 11 for OH^- and about 10 for H_3O^+ (the log *PZ* values are uncorrected for the H_2O concentration in the case of the acid catalysis; according to the mechanisms proposed, H_2O enters into the final rate equation only in the case of acid catalysis). When reactions occur between uncharged polar molecules (as in the NH_2OH case), the *PZ* factors are usually very small[21]; the smaller *PZ* value for the H_3O^+ reaction as compared with the OH^- reaction is very likely a manifestation of the higher degree of steric interference presented to the H_3O^+ ion as compared to the OH^- ion.[22]

Experimental

Preparation of Materials.—The simple thiolacetates were prepared by reaction of acetyl chloride or acetic anhydride with the mercaptan.[10,23] After washing with water and drying with sodium sulfate the colorless product was distilled from anhydrous potassium carbonate at atmospheric or reduced pressure. The middle fraction which was used boiled within a one-degree range. Acetylthiolglycolic acid was a commercial product redistilled. Acetylthiomalic acid was obtained in crystalline form by the use of thioacetic acid on maleic acid and thiomalic acid by hydrolysis of the acetylthiomalic acid.[24] NS-Diacetylcysteine was obtained in crystalline form from cysteine by acetylation with ketene.[25] N-Acetylcysteine was prepared by acetylation of L-cystine followed by reduction with zinc.[26]

Acid Hydrolysis.—To 100 ml. of temperature-equilibrated, accurately standardized 1 *N* HCl, about 2 microliters of thiolester, calculated to give an optical density of about 0.7 at 231 mμ, was added at zero time. A portion of the reaction mixture was transferred to a stoppered Beckman silica cell (1 *N* HCl was used as a blank). Optical density readings were taken frequently at first in order to extrapolate more accurately to the optical density at zero time. To minimize temperature errors part of the water from the thermostated bath was circulated through the cell housing of the Beckman DU spectrophotometer and between readings the cells were placed in the water-bath (temperature control of bath, ±0.05°).

Optical density measurements at 231 mμ were corrected for the absorption due to the mercaptan liberated by the formula

$$\text{O.D.}_{\text{mercaptan}} = \Delta\text{O.D.}\ \frac{\epsilon_{\text{mercaptan}}}{\epsilon_{\text{thiolester}} + \epsilon_{\text{mercaptan}}}$$

where ΔO.D. is the difference between the extrapolated zero time value of optical density and the optical density at time *t*.

The negative logarithms of the corrected optical densities were plotted against time in minutes to determine the apparent first-order rate constants.

Alkaline Hydrolysis.—About 10 microliters of thiolester, calculated to give an optical density at 231 mμ of about 0.7 when diluted with an equal volume of 0.012 *N* HCl, was added to redistilled, boiled water previously temperature equilibrated. Alkali, equivalent to the free carboxyl groups if any on the thiolester, was added. At zero time 25 ml. of temperature-equilibrated 0.100 *N* NaOH, prepared from standard 1 *N* NaOH, was added with rapid mechanical stirring (final volume 250 ml.). The course of the reaction was followed by removing, at suitable intervals, 5 ml. of reaction mixture with a rapid-flow pipet into 5-ml. aliquots of 0.012 *N* HCl. The acid essentially stopped the hydrolysis and quenched the absorption due to mercaptide ion. Measurements of the absorption at 231 mμ were corrected for the absorption due to mercaptan as in the acid hydrolysis rate studies. (The blank cell contained corresponding amounts of NaOH and HCl.) From the corrected absorption, the concentrations of thiolester were calculated and the initial concentration extrapolated. The concentrations of alkali were calculated from the initial concentration allowing for the consumption of two moles of alkali per mole of thiolester hydrolyzed as was done by previous workers in their rate studies.[9,10] However, because of the relatively high concentration of alkali compared to the thiolester, there was no significant difference in the second-order rate constant calculated on the basis of one or two moles of alkali consumed per mole of thiolester hydrolyzed.

Precautions were taken to minimize the volatility error with *n*-butyl thiolacetate by using a rubber-stoppered collapsible plastic reaction bottle. Samples were withdrawn through the rubber stopper by means of a syringe calibrated to deliver 2.9 ml. and were added directly to 0.1 ml. of 0.32 *N* HCl in a stoppered Beckman silica cell. By exerting a constant pressure on the plastic bottle, the head space was eliminated.

Reaction with Hydroxylamine.—The procedure followed was essentially the same as with the alkaline hydrolysis. The thiolester was added to temperature equilibrated water. At zero time 5 ml. of hydroxylamine reagent[27] was added (final volume 100 ml.). Samples of 5 ml. were withdrawn and added to 0.1 ml. of 0.55 *N* HCl before reading the optical density at 231 mμ. The blank cell contained the corresponding amounts of hydroxylamine reagent and hydrochloric acid. The optical density was corrected for the absorption due to liberated mercaptan as well as acethydroxamic acid ($\epsilon_{231\ \text{m}\mu}$ 50.7) by the formula

$$\text{O.D.}_{\text{mercaptan + acethydroxamic acid}} = \Delta\text{O.D.}\ \frac{\epsilon_{\text{mercaptan}} + \epsilon_{\text{acethydroxamic acid}}}{\epsilon_{\text{thiolester}} + \epsilon_{\text{mercaptan}} + \epsilon_{\text{acethydroxamic acid}}}$$

The optical density at zero time was obtained by extrapolation and the negative logarithms of the corrected optical density were plotted against time to determine the apparent first-order rate constants.

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[21] A. E. Remick, "Electronic Interpretations of Organic Chemistry," John Wiley and Sons, Inc., New York, N. Y., 1943, p. 367.

[22] As F. Daniels (*Ind. Eng. Chem.*, **35**, 504 (1943)) has pointed out, the frequency factors have long been a "catch-all" for hidden constants. In the case of acid catalysis, $k'' = k'[H_2O] = PZe^{-\Delta H^*/RT}$; $k' = (PZ/[H_2O])e^{-\Delta H^*/RT}$. The observed *PZ* factor should then be divided by *ca.* 55 molar to calculate the "absolute value"; or the *average* log $PZ \cong 10 - 1.74 \cong 8.3$, which is approximately 3 units lower than that of the OH^- case. If *Z*, the frequency factor, is assumed to be constant, then the differences lie in the entropy of activation, *i.e.*, the steric or probability factor, *P*.

[23] F. W. Wenzel, Jr., and E. E. Reid, THIS JOURNAL, **59**, 1089 (1937).

[24] B. Holmberg and E. Schjånberg, *Arkiv. Kemi, Mineral. Geol.*, **14A**, (No. 7), 9 (1940).

[25] A. Neuberger, *Biochem. J.*, **32**, 1452 (1938).

[26] N. W. Pirie and T. S. Hele, *ibid.*, **27**, 1716 (1933).

[27] 0.8 *M* hydroxylamine hydrochloride and 0.2 *M* hydroxylamine, prepared from recrystallized hydroxylamine hydrochloride and standard alkali.