KINETICS OF THE FORMATION OF SULFONIC ACIDS FROM DITHIO ACIDS. I

The Oxidation of Cystine to Cysteic Acid by Thallic Sulfate¹

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The mechanism of the oxidation and reduction reactions of sulfur compounds of the type R—S—S—R and R—SH is of interest because of the peculiar chemical reactions of these substances, their widespread occurrence in biological materials, and their supposed participation in the oxidation and reduction systems of the living organism.

When R—SH and R—S—S—R compounds are treated with sufficiently powerful oxidizing agents, the end product, if the organic radical is not attacked, usually is the corresponding sulfonic acid, R—SO₃H. The direct oxidation pathway might be represented as follows:

$$\mathbf{R} \longrightarrow \mathbf{SH} \xrightarrow{-2\mathbf{H}} \mathbf{R} \longrightarrow \mathbf{S} \longrightarrow \mathbf{S} \longrightarrow \mathbf{R} \xrightarrow{+\mathbf{O} + \mathbf{H}_2\mathbf{O}} 2(\mathbf{R} \longrightarrow \mathbf{SOH}) \xrightarrow{+\mathbf{O}} (\mathbf{R} \longrightarrow \mathbf{SO}_2\mathbf{H}) \xrightarrow{+\mathbf{O}} \mathbf{R} \longrightarrow \mathbf{SO}_3\mathbf{H}$$

The existence of the intermediates, R—SOH and R—SO₂H or their derivatives, for this series has not been demonstrated by stepwise oxidation of such sulfur compounds in aqueous solutions.

Recent investigations of the reactions of metallic salts with R—S—S—R demonstrate the formation of R—SO₃H and R—SH according to the following equation

 $3R-S-S-R + 3H_2O = 5R-SH + R-SO_3H$

This reaction appears to be an intramolecular oxidation-reduction driven by the removal of R—SH in the following series of hypothetical equilibria:

 $\begin{array}{l} R \longrightarrow S \longrightarrow R + H_2O \rightleftharpoons R \longrightarrow SH + R \longrightarrow SOH \\ R \longrightarrow SOH + H_2O + R \longrightarrow S \longrightarrow R \rightleftharpoons 2R \longrightarrow SH + R \longrightarrow SO_2H \\ R \longrightarrow SO_2H + H_2O + R \longrightarrow S \longrightarrow R \rightleftharpoons 2R \longrightarrow SH + R \longrightarrow SO_3H \end{array}$

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The R—SH may be removed by precipitation as a silver salt by Ag⁺ (6, 10), or as a mercuric salt by Hg⁺⁺ (2, 9), through formation of an unionized complex by mercuric halide ions BrHg⁺ (7), or through a combination oxidation and precipitation as a cuprous salt by Cu⁺⁺ (8). If the R—SH formed in the above reaction with water is not removed but instead is re-oxidized to R—S—S—R, there would be a continuous accumulation of R—SO₃H, which would ultimately appear as a complete conversion of R—S—S—R into R—SO₃H.

Since the possibility of isolating and identifying certain of the intermediates seems remote, an attempt has been made to obtain information concerning the mechanism of the reaction by kinetic measurements of the oxidation of R—S—S—R to R—SO₃H by various oxidizing agents.

The oxidation of cystine, $HOOC \cdot CHNH_2 \cdot CH_2S \cdot SCH_2 \cdot CHNH_2 \cdot COOH$, by thallic sulfate was selected, because with this oxidizing agent the reaction proceeds essentially quantitatively to cysteic acid, $HOOC \cdot CHNH_2 \cdot$ CH_2SO_3H and thallous sulfate. Thallic sulfate, further, forms with thallous sulfate a reversible oxidation-reduction system of known potential or oxidizing intensity, so that the results obtained may be useful for comparison with the data being obtained for other reversible oxidation-reduction systems.

EXPERIMENTAL

Preparation of reagents

Since chloride, bromide, and iodide were found to have unusual catalytic effects upon the reaction, special care was exercised in the selection of materials and preparation of solutions to insure that these substances would not be present in significant quantities.

Thallic oxide was suspended in 2.000 N sulfuric acid and dissolved by warming and concentrating to about 4 N. After proper dilution, the final solution was 0.005 gram-atomic in thallic sulfate and 1.000 N in sulfuric acid.

Cystine was prepared by the customary hydrochloric acid hydrolysis of hair and purified by several precipitations from sulfuric acid solution by sodium carbonate. The purity was checked by nitrogen determinations made by West and Brandon (11). The final solution was 0.01 M in cystine and 1.000 N in sulfuric acid.

Experimental procedures

The required amounts of sulfuric acid, water, and thallic sulfate were measured into a 250-cc. wide-mouth Pyrex flask which was immersed in a thermostat maintained within 0.1°C. At zero time the cystine solution was pipetted into the flask, which was shaken rapidly.

The reaction was stopped by suddenly adding 1 g. of potassium iodide in

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25 cc. of water. The excess thallic salt was determined by adding freshly prepared starch as indicator, and immediately titrating with 0.01 N sodium thiosulfate, drop by drop, in diffuse or artificial light. The color change was from a greenish blue, through yellowish green, to the final bright yellow suspension of precipitated thallous iodide. Before again using the reaction flasks, they were thoroughly cleaned with chromic–sulfuric acid mixture. Ten such experiments with different time intervals determined the reaction rate constant for the particular conditions.

Determination of the products of the reaction

Several typical experiments were made with 60 cc. of 0.005 gram-atomic thallic sulfate solution reacting with 5 cc. of 0.01 M cystine solution at 30°C. The amount of thallic salt reduced (see table 1) approaches a maximum of 10 oxidation-reduction equivalents per mole of cystine, which suggests the equation

 $\begin{array}{rl} 2\mathrm{HOOC}\cdot\mathrm{CHNH}_{2}\cdot\mathrm{CH}_{2}\mathrm{S}\cdot\mathrm{SCH}_{2}\cdot\mathrm{CHNH}_{2}\cdot\mathrm{COOH}\ +\ 5\mathrm{Tl}_{2}(\mathrm{SO}_{4})_{3}\ +\ 12\mathrm{H}_{2}\mathrm{O}\ =\ \\ 4\mathrm{HOOC}\cdot\mathrm{CHNH}_{2}\cdot\mathrm{CH}_{2}\mathrm{SO}_{3}\mathrm{H}\ +\ 5\mathrm{Tl}_{2}\mathrm{SO}_{4}\ +\ 10\mathrm{H}_{2}\mathrm{SO}_{4} \end{array}$

Experiments with the related dithiodihydracrylic acid, HOOC·CH₂. CH₂S·SCH₂·COOH, gave further indication that R—S—S—R type acids are oxidized almost entirely to R—SO₃H acids. Mixtures of 0.07317 g. of this acid as the disodium salt and 20 cc. of 0.174 N thallic perchlorate in N perchloric acid were allowed to react for 40 hours at 37°C. The Ba(·OOC·CH₂·CH₂·SO₃·) formed was isolated and analyzed according to a modification of the procedure previously reported (6). The R—SO₃H yield was 99 per cent of the theoretical and all except a trace of thallic salt was reduced (see table 2).

RESULTS OF KINETIC EXPERIMENTS

Mathematical considerations

The equation of the reaction indicates that 5 gram-atoms of thallic are reduced as 1 mole of cystine is oxidized. To permit the use of the less complex equations applying to pseudo first-order reactions, the cystine concentration was always made sufficiently high so that its initial concentration would change less than 10 per cent during the period of measurement. In calculating the reaction rate constants, only values between 20 and 80 per cent complete reaction were included and the arithmetical average of ten determinations, nearly equally spaced with respect to time, within this range, was considered sufficiently representative of the reaction rate under the particular conditions of the experiment. With but very few exceptions, the maximum variation of the constant in a particular series of ten values is less than 10 per cent. Table 3 gives the results of a typical series of determinations.

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TABLE 1

Determination of the maximum number of reducing equivalents of cystine towards thallic sulfate

MINUTES AT 30°C.	EQUIVALENTS OF THALLIC SULFATE PER MOLE OF	MINUTES AT 25°C.	EQUIVALENTS OF THALLIC SULFATE PER MOLE OF
			CYSTINE
30	4.652	840	9.800
60	6.464	840	9.900
120	8.142	2400	9,980
180	8.720	2400	10.002
240	9.242		
300	9.406		
480	9.780		
840	9.876		
1240	9.994		
1470	10.012		

TABLE 2

 $Quantity \ of \ sulfonic \ acid \ produced \ from \ dithiodihydracrylic \ acid \ by \ thallic \ sulfate$

Ba (·OOC·CH ₂ ·CH ₂ ·SO ₃ ·) ISOLATED	per cent Ba		
grams			
0.1982	47.0		
0.2010	47.0		
0.1992	47.0		
0.1979	47.2		
0.1995	47.1		
Av. = 0.1992	47.1		
Theor. $= 0.2015$	47.4		

TABLE 3

 $The results of a typical series of determinations \\ Temperature, 30°C.; thallic sulfate concentration 0.0005 gram-atomic; sulfuric acid \\ concentration, 0.5 N; cystine concentration, 0.002 M$

MINUTES	FRACTION OF THALLIC REDUCED	k (pseudo first order)	
3	0.263	0.102	
4	0.330	0.100	
5	0.418	0.108	
6	0.453	0.101	
7	0.524	0.106	
8.5	0.589	0.105	
10	0.650	0.105	
12	0.717	0.105	
14	0.761	0.102	
16	0.800	0.101	
rage	·····	0,104	

From the pseudo first-order constant calculated from the disappearance of thallic salt, the second-order constant may be calculated by dividing by the concentration of cystine. The value so obtained represents the rate at which the thallic salt was being reduced, calculating on a gram-atomic thallic and a molar cystine basis; the rate of cystine oxidation is only onefifth of this rate.

The results are summarized in tables 4 through 8. Each experiment numbered x is the same as some experiment listed in another table which has been inserted twice to preserve unity. Experiments lettered are inserted to show special effects of changing several variables.

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EXPERIMENT NO.	INI	TIAL CONCENTRAT	K	ĸ	
	Cystine	$Tl_2(SO_4)_3$	H_2SO_4	(PSEUDO FIRST ORDER)	(SECOND ORDER)
	gram-moles	gram-atoms Tl	gram-equivalents		
1	0.001	0.000125	1.0	0.0151	15.1
2	0.001	0.0001875	1.0	0.0177	17.7
3	0.001	0.00025	1.0	0.0197	19.7
4	0.001	0.000375	1.0	0.0220	22.0
5	0.001	0.0005	1.0	0.0234	23.4
6	0.001	0.001	1.0	0.0233	23.3
7	0.001	0.002	1.0	0.0227	22.7
А	0.001	0.00025	0.5	0.0478	47.8
$\mathbf{B}\mathbf{x}$	0.001	0.0005	0.5	0.0547	54.7
С	0.001	0.001	0.5	0.0564	56.4

TABLE 4

Effect of the concentration of the reactant, thallic sulfate, upon the rate of reaction at $30^{\circ}C$,

Thallic concentration effects

The values of K increase with an increase in thallic concentration until 0.0005 gram-atomic is reached; then nearly constant values are attained, showing that the reaction rate is probably proportional to the thallic concentration.

The oxidation-reduction potential values show similar changes (1), becoming less oxidizing as the thallic concentration is decreased below 0.0005 gram-atomic while keeping the thallic-thallous ratio the same. Whether the potential and rate changes are related remains for further work to decide.

Cystine concentration effects

As the concentration of cystine is increased there is a slight decrease in K (see table 5). At values above 0.002 molar, K becomes nearly constant,

so that the reaction rate may be considered proportional to the cystine concentration.

EXPERIMENT	IN	ITIAL CONCENTRATI	K	<i>K</i> *		
NO.	Cystine	Cystine Tl ₂ (SO ₄);		(PSEUDO FIRST ORDER)	(SECOND ORDER)	
	gram-moles	gram-atoms Tl	gram-equivalents			
11	0.0005	0.0005	1.0	0.0123	24.6	
12	0.00075	0.0005	1.0	0.0169	22.7	
13x	0.001	0.0005	1.0	0.0234	23.4	
14	0.002	0.0005	1.0	0.0420	21.0	
15	0.003	0.0005	1.0	0.0600	20.0	
16	0.004	0.0005	1.0	0.0791	19.8	
17	0.005	0.0005	1.0	0.097	19.4	
21x	0.001	0.001	1.0	0.0233	23.3	
22	0.002	0.001	1.0	0.0422	21.1	
23	0.003	0.001	1.0	0.0625	20.8	
24	0.004	0.001	1.0	0.0807	20.2	
25	0.005	0.001	1.0	0.103	20.6	
D	0.0005	0.0005	0.5	0.0294	68.8	
$\mathbf{E}\mathbf{x}$	0.001	0.0005	0.5	0.0547	54.7	
F	0.002	0.0005	0.5	0.104	52.0	

TABLE 5

 $Effect \ of \ the \ concentration \ of \ the \ reactant, \ cystine, \ upon \ the \ rate \ of \ reaction \ at \ 30^\circ C.$

TABLE 6

Effect of the concentration of the product, thallous sulfate, upon the rate of reaction at $30^{\circ}C$.

EXPERIMENT	INITIAL CONCENTRATIONS				K	K
NO.	Cystine	Tl ₂ (SO ₄)3	Tl ₂ SO4	H_2SO_4	(PSEUDO FRST ORDER)	(SECOND ORDER)
	gram-moles	gram-atoms Tl	gram-atoms Tl	gram-equiva- lents		
31	0.001	0.0005	0.0025	1.0	0.0237	23.7
31x	0.001	0.0005		1.0	0.0234	23.4
32	0.001	0.00025	0.0025	1.0	0.0204	20.4
32x	0.001	0.00025		1.0	0.0197	19.7
33	0.001	0.000375	0.001875	1.0	0.0233	23.3
33x	0.001	0.000375		1.0	0.0220	22.0
34	0.001	0.0005	0.005	0.5	0.0552	55.2
34x	0.001	0.0005		0.5	0.0547	54.7

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Thallous sulfate concentration effects

The lack of significant change in the ten pseudo first-order constants obtained in a particular experiment and the close agreement between the values of K (see table 6) for experiments with and without initially added

EXPERIMENT NO.	IN	ITIAL CONCENTRAT	K	k'	
	Cystine Tl ₂ (SO ₄) ₃		H ₂ SO ₄	(PSEUDO FIRST ORDER)	(SECOND ORDER
	gram-moles	gram-atoms Tl	gram-equivalents		
41	0.001	0.0005	0.25	0.143	143.0
42	0.001	0.0005	0.375	0.0918	91.8
44	0.001	0.0005	0.5	0.0547	54.7
44	0.001	0.0005	0.75	0.0335	33.5
45	0.001	0.0005	1.0	0.0238	23.8
4 6	0.001	0.0005	1.25	0.0163	16.3
47	0.001	0.0005	1.5	0.0135	13.5
48	0.001	0.0005	1.8	0.0113	11.3

TABLE 7

Effect of the concentration of sulfuric acid upon the rate of reaction at 30° C.

TABLE 8

Effect of temperature on the rate of reaction at different acidities

EXPERIMENT NO.	TEMPERATURE	INI	TIAL CONCENTRAT	K	K	
		Cystine	$Tl_2(SO_4)_3$	H_2SO_4	(PSEUDO FIRST ORDER)	(SECOND ORDER)
	degrees C.	gram-moles	gram-atoms Tl	gram- equivalents		
1 T	15	0.001	0.0005	0.25	0.0171	17.1
$2 \mathrm{T}$	15	0.001	0.0005	0.5	0.00720	7.20
3 T	20	0.001	0.0005	0.25	0.0319	31.9
4 T	20	0.001	0.0005	0.5	0.0137	13.7
5 T	20	0.001	0.0005	1.0	0.00587	5,87
6 T	25	0.001	0.0005	0.25	0.0709	70.9
$7 \mathrm{T}$	25	0.001	0.0005	0.5	0.0287	28.7
8 T	25	0.001	0.0005	1.0	0.0117	11.7
9 Tx	30	0.001	0.0005	0.25	0.143	143.0
10 Tx	30	0.001	0.0005	0.5	0.0547	54.7
11 Tx	30	0.001	0.0005	1.0	0.0238	23.8
12 T	35	0.001	0.0005	0.5	0.100	100.0
13 T	35	0.001	0.0005	1.0	0.0404	40.4

thallous, indicate that thallous concentration and consequently also the oxidation-reduction potential of the thallic-thallous system have no apparent effect on the reaction rate.

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Sulfuric acid concentration effect

The reaction rate decreases with an increase in sulfuric acid concentration (see table 7). No simple relation could be found between the rate and several of the usual methods of expressing acidities, and considering that cystine has two acidic and two basic ionizations in or near the region of acidity used, this is not surprising.

Temperature effects

The reaction rates at three acidities were determined (see table 8), and the graph of the reciprocal of the absolute temperature plotted against the logarithm of K results in three parallel lines whose slope is such that Q = 24,200 calories in the Arrhenius equation (3).

DISCUSSION AND SUMMARY

The kinetic experiments performed indicate that the rate-controlling step in the measured reaction between thallic sulfate and cystine in sulfuric acid solution is a second-order reaction.

The rate of reaction is approximately proportional to the cystine concentration and the thallic sulfate concentration and decreases with an increase in the sulfuric acid concentration. The equation for the complete reaction is

$$\begin{array}{rl} 2\mathrm{HOOC}\cdot\mathrm{CHNH}_{2}\cdot\mathrm{CH}_{2}\mathrm{S}\cdot\mathrm{SCH}_{2}\cdot\mathrm{CHNH}_{2}\cdot\mathrm{COOH} \ + \ 5\mathrm{Tl}_{2}(\mathrm{SO}_{4})_{3} \ + \ 12\mathrm{H}_{2}\mathrm{O} \ = \\ & 4\mathrm{HCOC}\cdot\mathrm{CHNH}_{2}\cdot\mathrm{CH}_{2}\mathrm{SO}_{3}\mathrm{H} \ + \ 5\mathrm{Tl}_{2}\mathrm{SO}_{4} \ + \ 10\mathrm{H}_{2}\mathrm{SO}_{4} \end{array}$$

and indicates that 5 gram-atoms of thallic are reduced as 1 gram-mole of cystine is oxidized. At 30°C. in 1 N sulfuric acid with 0.0005 gramatomic thallic sulfate and 0.001 M cystine, the value of the second-order constant for thallic reduction is $K_{\rm Tl} = 23.3$ gram-atoms per minute per liter; for cystine oxidation under the same conditions $K_{\rm Cys.} = 4.7$ moles per minute per liter.

Since several second-order reactions are possible in the series leading from R—S—S—R to R—SO₃H, the identification of a particular reaction is not possible with the data at hand. However, two reactions can be eliminated as being involved in the rate-controlling step with thallic salt as oxidant: the reaction of R—S—S—R with H₂O to give R—SH and R—SOH, since this would result in the reaction being independent of the concentration of the thallic salt; and the oxidation by thallic salt of R—SH formed by hydrolysis, since this oxidation would probably not be a simple bimolecular reaction. The approximate constancy of $K_{\text{pseudo first order}}$ in a particular series and the lack of an appreciable effect upon the rate of reaction upon the addition of thallous salt practically eliminates the possibility that the rate-controlling step involves a labile, reversible, electromotively active oxidation-reduction system (4, 5) composed of intermediate oxidation stages of the sulfur compounds.

The energy of activation, Q, was found to be 24,200 calories at various acidities.

The oxidation of R—S—S—R to R—SO₃H by various oxidizing agents is being studied in the hope of solving the problem of the mechanism of this complex oxidation.

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