

correlated with other activity measurements of various types for four other solvents and a general discussion has been given of the value of such cor-

relations in comparing the basic strengths of different solvents.

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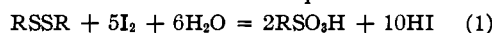
[CONTRIBUTION FROM THE JOHN HARRISON LABORATORY OF THE UNIVERSITY OF PENNSYLVANIA]

A Kinetic Study of the Oxidation of Cystine by Iodine¹

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Introduction

Real understanding of the physiological significance of organic sulfur compounds depends largely upon a knowledge of their oxidation processes. Although the study of the oxidation of cysteine (or thiol compounds in general) has been thorough,³ the oxidation of cystine (or disulfides in general) has received little attention. One of the authors made a brief report⁴ on the oxidation of cystine by iodine, following the establishment⁵ of the stoichiometric equation



Here it was pointed out that the reaction expressed by equation (1) probably takes place as a series of consecutive bimolecular reactions. In the present paper it will be shown that the rate-determining step in the series of reactions represented in (1) is the reaction between a molecule of cystine and a molecule of iodine or substances whose concentrations are proportional to their concentrations. Moreover, the effect of sodium chloride and of hydrogen and iodide ions will be shown.

Experimental Part

I. Purification of Cystine.—*l*-Cystine from Merck and Company was purified by the method of Gerwe⁶ with a slight modification in the final stage. The modification consisted of suspending the *l*-cystine hydrochloride in redistilled water, dissolving it by introducing hydrogen chloride gas, and neutralizing with ammonia gas, upon which an abundant precipitate of *l*-cystine was obtained. The original *l*-cystine was found to contain $4 \times 10^{-3}\%$ iron when analyzed by the method of Lachs and Friedenthal.⁷ The iron content was reduced by the purification

(1) This paper is abstracted from the first part of the thesis presented in April, 1933, by Kamenosuke Shinohara to the Faculty of the Graduate School of the University of Pennsylvania, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

(2) Robert McNeil Fellow at the Research Institute of the Lankenau Hospital, Philadelphia, Pa. The experimental work was carried out at the Institute.

(3) Ghosh, Raychandhuri and Ganguli, *J. Indian Chem. Soc.*, **9**, 43 (1932); **9**, 53 (1932); Elliot, *Biochem. J.*, **24**, 310 (1930); Elvehjem, *ibid.*, **24**, 415 (1930).

(4) Shinohara, *J. Biol. Chem.*, **97**, XII (1932).

(5) Shinohara, *ibid.*, **96**, 285 (1932).

(6) Gerwe, *J. Biol. Chem.*, **91**, 57 (1931).

(7) Lachs and Friedenthal, *Biochem. Z.*, **33**, 130 (1911).

to $4 \times 10^{-5}\%$, which is practically the amount found in the hydrochloric acid used for the color test. The specific rotation $[\alpha]_{5461}^{25}$ of a 1% solution of *l*-cystine in *N* hydrochloric acid was -248.5° for the original cystine, and -250.7° for the purified material. The latter value is practically the maximum rotation obtainable for *l*-cystine.⁸

II. Technique.—Since even in acid solution cystine decomposes slowly on standing to produce cysteine,⁹ it was necessary to make up a fresh solution of *l*-cystine for each experiment. *l*-Cystine was first dissolved in 2 *M* hydrochloric acid, and water was added until the solution was 0.01 *M* in *l*-cystine and 0.2 *M* in hydrochloric acid. To a 500-cc. flask containing the *l*-cystine and other constituents (excepting iodine) at $25 \pm 0.01^\circ$, the desired volume of iodine solution in 4% potassium iodide was added, and the flask was filled to the mark with water and shaken. At intervals, 25 cc. of solution was pipetted out and run into a volume of standard sodium thiosulfate solution slightly less than that required for titration of the iodine present. The titration was then completed quickly by addition of sodium thiosulfate solution from the buret.

In order to correct for loss of iodine due to volatilization, reaction with impurities in the acetic acid used for buffers, etc., a blank experiment was run each time. The composition of the blank solution was the same as that of the reaction mixture except that an equivalent amount of *dl*-alanine was substituted for *l*-cystine. The consumption of iodine by cystine was calculated from the difference between the values obtained by titration of the blank and test solutions.

III. Determination of the Extent and Order of the Reaction.—To determine to what extent the stoichiometric equation (1) holds, two experiments were carried out, one in a sodium acetate-acetic acid buffer,¹⁰ one in

(8) Toennies and Lavine, *J. Biol. Chem.*, **89**, 153 (1930).

(9) Shinohara and Kilpatrick, *ibid.*, **105**, 241 (1934).

(10) According to Sano [*Biochem. Z.*, **168**, 29 (1926)] the solubility of *l*-cystine at *P_H* 3.0–6.0 is 4.64×10^{-4} *M* at 25°. However, upon dilution or neutralization of a solution of cystine in acid, precipitation does not occur for some time even though the concentration of cystine exceeds the solubility. Nor does seeding with a crystal of cystine start crystallization. Solutions 5, 8 and 10×10^{-4} *M* in *l*-cystine, in a buffer solution of the composition CH_3COONa 0.40 *M*, CH_3COOH 0.40 *M*, KI 0.024 *M*, HCl 0.060 *M*, remained clear for at least one week. A solution originally 12×10^{-4} *M* in *l*-cystine in the above buffer showed a very slight precipitate on the second day, and a solution originally 20×10^{-4} *M*, a precipitate after four hours. That the salt concentration has small effect upon the solubility was demonstrated by experiments in buffer solutions 0.4, 0.2 and 0.02 *M*, respectively, in sodium acetate and acetic acid. An excess of cystine was shaken with the solution at $25 \pm 1^\circ$ for three days, and upon analysis at the end of a week, the solutions were found to contain 5.2, 5.4 and 5.7×10^{-4} moles of cystine per liter, respectively.

0.1 *N* hydrochloric acid solution. The results are given in Table I.

TABLE I

REACTION OF IODINE WITH <i>l</i> -CYSTINE							
Composition of the reaction mixture (moles per liter $\times 10^4$)							
Expt.	<i>l</i> -Cystine	I ₂	KI	HCl	KCl	HAc	NaAc
A	10.00	99.53	482	200	1000	4000	4000
B	10.00	99.57	482	1200	4000		

Temperature, $25 \pm 0.01^\circ$. u = moles I₂ reacted per liter $\times 10^4$. k = velocity constant, in liters per mole per minute.

Time in hours	A		B	
	u	k	u	k
3.00	15.98	1.178	16.99	1.278
6.00	22.55	1.174	27.05	1.293
9.00	36.02	1.189	33.51	1.306
24.0	44.84	1.172	45.65	1.281
27.0	45.94	1.179	46.43	1.253
30.0			47.34	1.282
32.0	47.06	1.153		
48.0	49.42	1.322 ^a	49.12	1.177 ^a
51.0	Av.	1.174	49.23	1.151 ^a
54.0	49.76 a. d.	0.008		
72.0	50.30		49.43	0.884 ^a
79.7	50.56		Av.	1.282
80.0			49.49 a. d.	0.012
95.6	50.74			
96.0	50.76		49.53	
104.0			49.49	
120.0			49.57	

^a Omitted from average.

According to equation (1), the amount of iodine required for reaction with the *l*-cystine is 50.00×10^{-4} mole per liter. The data show that the amount reacted in A slightly exceeds, while the amount reacted in B does not quite reach the theoretical amount. At the end of four days the reaction mixtures were tested for sulfate; sulfate of approximate concentration 0.6×10^{-4} *M* was found in A, while none was detected in B. The presence of sulfate in the solution of lower hydrogen-ion concentration may be attributed to the oxidation by iodine of the products of the hydrolysis of cystine.⁹ Since the amount of iodine consumed by the side reaction is so small in the buffer solution, and undetectable in the solution of hydrochloric acid, one may conclude that under the experimental conditions in question the reaction between cystine and iodine proceeds almost quantitatively to the cysteic acid stage.

If the rate-determining step in the oxidation of cystine by iodine is the reaction between a molecule of cystine and a molecule of iodine (or substances whose concentrations are proportional to the concentrations of cystine and iodine), the equation

$$\frac{du}{dt} = k \left(a - \frac{u}{5} \right) (b - u) \quad (2)$$

where u is the number of moles of iodine reacted per liter at time t , a the initial molar concentration of cystine, and b of iodine, should be valid. If the initial concentrations of cystine and iodine are equivalent, *i. e.*, if $a = b/5$, (2) yields upon integration

$$\frac{u_2(b - u_1)}{u_1(b - u_2)} = \frac{t_2}{t_1} \quad (3)$$

To test the applicability of (2) and (3), two experiments with equivalent initial concentrations were performed. The results are given in Table II.

TABLE II

THE REACTION OF CYSTINE WITH IODINE						
Composition of the reaction mixture (moles per liter $\times 10^4$)						
Expt.	<i>l</i> -Cystine	I ₂	KI	HCl	HAc	NaAc
A	10.00	50.12	400	600	4000	4000
B	5.00	25.00	120.5	600	4000	4000

Temperature, $25 \pm 0.01^\circ$. u = moles I₂ reacted per liter $\times 10^4$.

Time in minutes	u		t_2/t_1	$\frac{u_2(b - u_1)}{u_1(b - u_2)}$	
	A	B		A	B
15	0.84	0.29			
30	1.79	.51			
60	3.35	.88	60/30 = 2	1.93	1.75
90	4.79	1.28			
120	6.20	1.66	120/60	1.97	1.95
150	7.55	1.98			
180	8.73	2.38	189/90	2.00	1.95
210	9.85	2.69			
240	10.98	3.01	240/120	1.99	1.92
300	12.98	3.63	300/150	1.97	1.97
360	14.84	4.18	360/180	1.99	1.91
420	16.43	4.67	420/210	2.00	1.91

The integration of (2) yields for the general case

$$k = \frac{2.303}{i(a - b/5)} \log \frac{b(a - u/5)}{a(b - u)} \quad (4)$$

In the experiments of Table I, the reaction was followed over practically its entire course. When the data of Table I are substituted in (4), the velocity constants listed in the third and fifth columns of that table are obtained. If the determinations are omitted in which $(a - u/5)$ is very small, and the probable error accordingly large, the velocity constant is constant throughout the reaction. The difference between the velocity constants obtained in Experiments A and B is due to the difference in medium.

In Table III are given the results of a series of experiments in which the concentrations of reactants were varied. The reaction was followed for seven hours, and the constants listed are average values of the constants obtained after

the first forty-five minutes of the reaction. The hydrogen-ion concentration of the buffer solutions is $4 \times 10^{-5} M$, and that of the solution of Experiment C, 0.09 M .

TABLE III

REACTION OF IODINE WITH *l*-CYSTINE

Temperature, $25 \pm 0.01^\circ$. k = velocity constant in liters per mole per minute.

Expt.	Composition of the reaction mixture, moles per liter $\times 10^4$						k	a. d.
	<i>l</i> -Cystine	I ₂	HCl	KI	HAc	NaAc		
A ₁	10.00	24.98	600	120	4000	4000	1.145	0.021
A ₂	5.00	24.98	600	120	4000	4000	1.152	.028
B ₁	10.00	100.0	600	482	4000	4000	1.171	.007
B ₂	10.00	75.38	600	482	4000	4000	1.156	.002
B ₃	10.00	50.10	600	482	4000	4000	1.174	.002
B ₄	10.00	25.06	600	482	4000	4000	1.155	.020
B ₅	10.00	12.53	600	482	4000	4000	1.191	.015
C	20.00	24.80	900	120.5	0	NaCl 4000	1.155	.005

The average value of the velocity constant for the experiments in sodium acetate-acetic acid buffer solution given in Table III is 1.163, a. d. 1%. These experiments, together with those of Tables I and II, establish (2) as the equation governing the rate of the reaction.

IV. Effect of Light, and of Ferric and Cupric Ions at Low Concentration.—Duplicate experiments performed in tinfoil-covered flasks and in unshielded flasks showed no difference greater than the error of measurement.

Ferric chloride and ferric alum at concentrations below $10^{-4} M$ showed no effect. At higher concentrations, oxidation of iodide ion by the ferric ion was observed. As further proof that small amounts of iron have no marked catalytic effect, it may be stated that three samples of *l*-cystine, of iron content 0.12%, $4 \times 10^{-3}\%$, and $4 \times 10^{-5}\%$, respectively, were oxidized by iodine at the same rate. The study of the effect of copper salts upon the rate of oxidation is complicated by the precipitation, on standing, of a copper salt of cystine.¹¹ The initial rate of reaction in a solution $2 \times 10^{-4} M$ in cupric acetate was, however, found the same as the initial rate in a solution without copper salt.

V. The Effect of Sodium Chloride.—The production of hydriodic acid in the oxidation of cystine by iodine furnishes an obstacle to the study in dilute solution of the effect of added salts. As will be shown in later sections, the velocity decreases with increase in hydrogen-ion concentration and increases with increase in iodide concentration in the range 0.01 to 0.06 M iodide. In the experiments designed to measure

the salt effect, the concentration of strong acid must therefore be such that the hydriodic acid formed causes only a relatively small change in hydrogen-ion concentration. This precludes the possibility of determining from the salt effect the charge type of the reaction.

In Table IV are given the results of a series of experiments in which, by addition of sodium chloride, the ionic strength was increased from 0.1 to 2. The solutions were made 0.09 M in hydrochloric acid, so that the increase in hydrogen-ion concentration in the seven-hour period of observation, during which approximately one-half of the iodine was converted to hydriodic acid, was less than 4%. In computing the ionic strength, the presence of cystine ions of single and double positive charge,¹² and the formation of hydriodic acid, were neglected. Lack of knowledge of the dissociation constants of cystine in the salt solutions employed makes the former correction doubtful. The velocity constant, in the fourth column of the table, is the average of the values obtained after the first forty-five minutes of the reaction.

TABLE IV

EFFECT OF SODIUM CHLORIDE

Temperature, $25 \pm 0.01^\circ$. k = velocity constant in liters per mole per minute. Composition of reaction mixture in moles per liter $\times 10^4$: *l*-cystine, 20.00; I₂, 24.80; KI, 120.5; HCl, 900; NaCl as shown.

Expt.	NaCl	$\mu \times 10^4$	k	a. d.
A	0	1020	1.611	0.007
B	500	1520	1.477	.006
C	1000	2020	1.399	.003
D	2000	3020	1.285	.008
E	4000	5020	1.154	.008
F	5000	6020	1.111	.008
G	10000	11000	1.004	.004
H	20000	21000	0.962	.007

For the range of ionic strength 0.1 to 0.5 these results may be expressed with an accuracy of 2% by the equation

$$\log k = 0.373 - 0.609 \sqrt{\mu} + 0.226 \mu \quad (5)$$

obtained by the method of least squares.¹³

VI. The Effect of Hydrogen Ion.—To determine the effect of the hydrogen ion, a series of experiments was carried out at constant ionic strength, and with hydrogen-ion concentrations ranging from 0.03 to 0.6 M . The hydrogen-ion concentration of each solution was taken as equal to the stoichiometric concentration of hydro-

(12) Cannan and Knight, *Biochem. J.*, **21**, 1384 (1927).

(13) The computations were made by Miss Ethel P. Echternach, under the guidance of Dr. Frederick H. Safford.

(11) Mauthner, *Z. für. Biol.*, **42**, 184 (1901).

chloric acid. Since the dissociation constants of cystine in these solutions are not known, correction cannot be made with sufficient accuracy for the hydrogen ions combined with cystine. This correction is necessarily small, and is partially offset by the formation of hydriodic acid. A constant ionic strength of 0.60 was maintained by addition of sodium chloride. The experiments were carried out in the dark. The blank experiments showed that the production of iodine from hydriodic acid was negligible under the conditions employed, the increase in iodine concentration in even the most acid solution being less than 1% in six hours. The reaction was followed for about seven hours, and the velocity constant in the fourth column of Table V represents the average of the values obtained after the first forty-five minutes. In the case of the four most strongly acid solutions, F, G, H and I, the velocity constant showed a tendency to increase with time, the magnitude of the increase in the period from $t = 60$ to $t = 360$ minutes being 3, 4, 5 and 6%, respectively.

TABLE V
EFFECT OF HYDROGEN ION

Temperature, $25 \pm 0.01^\circ$. k = velocity constant in liters per mole per minute. Composition of the reaction mixture in moles per liter $\times 10^4$: *l*-cystine, 20.00; I_2 , 24.76; KI, 120.5; HCl and NaCl as shown.

Expt.	HCl	NaCl	k	a. d.
A	300	5600	1.129	0.006
B	400	5500	1.120	.006
C	650	5250	1.115	.005
D	900	5000	1.110	.003
E	1900	4000	1.084	.009
F	2900	3000	1.063	.011
G	3900	2000	1.042	.011
H	4900	1000	1.025	.012
I	5900	0	0.992	.016

The velocity constants of Table V may be expressed by

$$k = 1.129 - 0.228 C_{H_3O^+} \quad (6)$$

In Table VI are given the results of some experiments in sodium tri-, di- and monochloroacetate solutions.

The hydrogen-ion concentration of the trichloroacetate solutions was 0.050 *M*, that of the

dichloroacetate solution approximately 0.002 *M*, and that of the monochloroacetate solution approximately 0.0004 *M*.

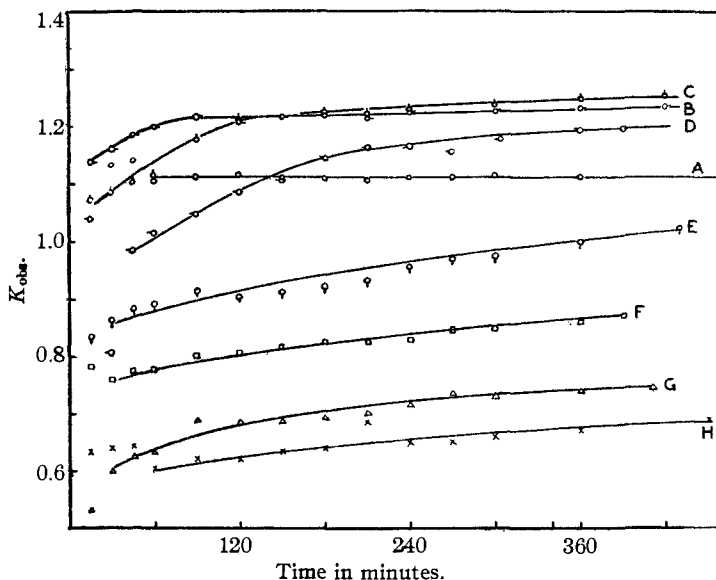


Fig. 1.—Composition of reaction mixture in moles per liter $\times 10^4$: *l*-cystine, 20.00; I_2 , 24.80; HCl, 900; KI and NaCl as shown.

Expt.	Symbol	NaCl	KI	Expt.	Symbol	NaCl	KI
A	○	5000	120	E	◊	3000	2120
B	◊	4800	320	F	□	2000	3120
C	◊	4500	620	G	△	1000	4120
D	◊	4000	1120	H	×	0	5120

TABLE VI

EXPERIMENTS IN SODIUM TRI-, DI- AND MONOCHLORO-ACETATE SOLUTIONS

Temperature, $25 \pm 0.01^\circ$. k = velocity constant (average of values obtained after forty-five minutes) in liters per mole per minute. Composition of reaction mixture in moles per liter $\times 10^4$: *l*-cystine, 20.00; I_2 , 24.76; KI, 120; HCl, 500; NaCl and acetates as shown.

Expt.	NaCl	Acetate	k	a. d.	
A ₁	1000	CCl ₃ COONa	4000	1.205	0.026
A ₂	3000	CCl ₃ COONa	2000	1.169	.008
B	1000	CHCl ₂ COONa	4000	1.194	.002
C	1000	CH ₂ ClCOONa	4000	1.140	.003

VII. The Effect of Iodide.—Because of the fact that with reactions involving the halogens the rate is frequently determined by a reaction in which the corresponding hypohalous acid takes part,¹⁴ it is of interest to examine the effect of iodide upon the reaction between cystine and iodine.

The results of a series of experiments designed to test the effect of iodide are shown in Fig. 1. An ionic strength of 0.60 was maintained throughout the series by the use of sodium chloride. The experiments were performed in flasks covered

(14) W. C. Bray, *Z. physik. Chem.*, **54**, 465 (1906).

with tinfoil. The blanks showed that in solutions of iodide concentration less than 0.06 *M* there was no observable increase in iodine concentration on standing, while at higher concentrations the rate of formation of iodine was proportional to the iodide concentration. In the case of the solution 0.5 *M* in potassium iodide (the highest concentration employed) the iodine concentration increased by 4% in six hours. As in all the previous experiments, the amount of iodine reacted with cystine was taken as the difference between the amounts present in the blank and test solutions at a given time. In Fig. 1 the velocity constant is plotted against the time elapsed.

The velocity constant increases with the iodide concentration in the range 0.01–0.06 *M* iodide. It is evident that in the experiments at iodide concentration 0.06 *M* and above (C, D, E, F, G, H) the velocity constant tends to increase with time. The same tendency, to a lesser degree, was observed in the experiments at hydrogen-ion concentrations above 0.2 *M*.

VIII. Experiments with Bromine and Chlorine.—Experiments were carried out in which iodine was replaced by bromine, the technique being the same as that already described except that before titration the sample of the reaction mixture was added to a sufficient amount of potassium iodide solution, after which the iodine liberated was titrated with sodium thiosulfate. The reaction between cystine and bromine is practically instantaneous. Qualitative experiments indicated that the reaction of cystine with chlorine is instantaneous also.

Discussion

The problem of determining the reactants in the rate-determining step of the oxidation of cystine by iodine is complicated by the fact that cystine in acid solution exists in three forms, the neutral molecule or zwitterion R_0 , and the ions RH^+ and RH_2^{++} . According to the dissociation constants given by Cannan and Knight,¹² at *PH* 5 cystine exists as the neutral molecule, and as the hydrogen-ion concentration increases there appears the ion RH^+ and, later, RH_2^{++} . Change in hydrogen-ion concentration (and to some extent change in salt concentration) by altering the percentages of the three forms may accordingly bring about change in the rate of oxidation of cystine by iodine. If it be assumed that

equilibrium between the three forms is always maintained, in a constant medium the velocity of the reaction is given by the equation

$$\begin{aligned} -\frac{d(R)}{dt} &= -\frac{d(I_2)}{dt} = \\ &= [k_0(R_0) + k_1(RH^+) + k_2(RH_2^{++})](I_2) \\ &= (R_0)(I_2)[k_0 + k_1K_1(H_3O^+) + k_2K_1K_2(H_3O^+)^2] \\ &= (R)(I_2) \left[\frac{k_0 + k_1K_1(H_3O^+) + k_2K_1K_2(H_3O^+)^2}{1 + K_1(H_3O^+) + K_1K_2(H_3O^+)^2} \right] \end{aligned}$$

where the parentheses () signify "concentration of," when *R* represents the total cystine, where k_0 , k_1 and k_2 are the velocity constants for the reaction with iodine of R_0 , RH^+ and RH_2^{++} , respectively, and where

$$\begin{aligned} K_1 &= (RH^+)/(R_0)(H_3O^+) \\ K_2 &= (RH_2^{++})/(RH^+)(H_3O^+) \end{aligned}$$

Letting

$$k = \frac{k_0 + k_1K_1(H_3O^+) + k_2K_1K_2(H_3O^+)^2}{1 + K_1(H_3O^+) + K_1K_2(H_3O^+)^2}$$

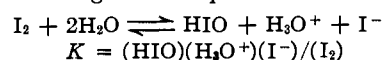
it is evident that (a) if $k_1 = k_2 = 0$, with increasing (H_3O^+) k must decrease and by the same percentage as $(R_0)/(R)$ decreases. (b) If $k_0 = k_1 = k_2$, k equals k_0 and remains constant as (H_3O^+) increases. (c) If $k_1 > k_0$, $k_2 > k_0$, then $k > k_0$ by an amount depending on k_0 , k_1 , k_2 , K_1 , K_2 and (H_3O^+) .

Comparing experiments at constant iodide concentration, and approximately constant ionic strength, it is evident that over a wide range of

Expt.	(H_3O^+)	μ	k
A ₁ , A ₂ , Table III	4×10^{-2}	0.4 (mostly CH_3COONa)	1.15
C, Table VI	4×10^{-2}	.5 (mostly $CH_2ClCOONa$)	1.14
B, Table VI	2×10^{-2}	.5 (mostly $CHCl_2COONa$)	1.19
C, Table III; E, Table IV	9×10^{-2}	.5 (mostly $NaCl$)	1.15

hydrogen-ion concentration, in which there is presumably wide variation in the ratio $(R_0)/(R)$, there is small change in the velocity constant. The change observed in the velocity constant with change in hydrogen-ion concentration at a constant ionic strength of 0.60 (Table V) might very well be a "second order" salt effect.

Hypiodous acid does not appear to be involved in the rate-determining step of the oxidation of cystine by iodine. If the rate-determining step were the reaction between cystine and hypiodous acid, the latter in equilibrium with iodine according to the equation



we should have

$$\begin{aligned} -d(I_2)/dt &= k'(HIO)(cystine) = \\ &= k'(I_2)(cystine)/(H_3O^+)(I^-) \\ &= k(I_2)(cystine) \text{ where } k = k'K/(H_3O^+)(I^-) \end{aligned}$$

Upon doubling the hydrogen-ion or iodide concentration, then the observed velocity constant should be halved. Experiments at constant iodide concentration and ionic strength show much smaller decrease in the velocity constant with increase in hydrogen-ion concentration than required by this hypothesis (see Table V). And as Fig. 1 shows, with increase in iodide concentration at constant hydrogen-ion concentration and ionic strength, there is an increase in the velocity constant in the range from 0.01 to 0.06 *M* iodide.

All the solutions in which the oxidation of cystine by iodine was studied were of *PH* less than 5, and contained iodide and chloride ions. The hydrolysis of iodine in solutions of *PH* less than 5 is slight, and it may be considered that all the iodine was present as I_2 , I_3^- and I_2Cl^- .

Assuming that the equilibria



are maintained, in a constant medium the velocity of the reaction is given by the equation

$$\begin{aligned} -d(I_2, \text{tot.})/dt &= [k_0(I_2) + k_1K_1(I_2)(I^-) + \\ &\quad k_2K_2(I_2)(Cl^-)] (\text{cystine}) \\ &= (I_2)(\text{cystine})[k_0 + k_1K_1(I^-) + k_2K_2(Cl^-)] \\ &= (I_2, \text{tot.})(\text{cystine}) \left[\frac{k_0 + k_1K_1(I^-) + k_2K_2(Cl^-)}{1 + K_1(I^-) + K_2(Cl^-)} \right] \end{aligned}$$

where the parentheses () signify "concentration of," where $I_2, \text{tot.} = I_2 + I_3^- + I_2Cl^-$, and where k_0 , k_1 and k_2 are the velocity constants for the reaction of I_2 , I_3^- and I_2Cl^- , respectively, with cystine. In pure water, $1/K_1$ is 0.00140¹⁵ and $1/K_2$ 0.629.¹⁶ Spivey and Dawson¹⁷ and La Mer and Lewinsohn¹⁶ found that in potassium chloride solution the equilibrium constant $1/K_1$ tends to decrease with increasing iodide concentration. The average of their values for $1/K_1$ in 1.65 *M* potassium chloride solution containing from 0.01 to 0.1 mole of iodide per liter is 0.00157, the decrease observed with increasing iodide concentration amounting to about 10% in the range specified. In 1.65 *M* potassium chloride solution $1/K_2$ is 0.658.¹⁶ For solutions of ionic strength 0.60 (mostly sodium chloride), and iodide concentration between 0.01 and 0.1 *M*, let us take $1/K_1$ as 0.00146 and $1/K_2$ as 0.640. In Table VII are given the concentrations of I_2 , I_3^- and I_2Cl^- , computed from these constants, for the first three experiments of Fig. 1. The stoi-

chiometric concentration of iodide was taken as equal to the concentration of potassium iodide in each case, plus 0.0025, the amount of hydriodic acid formed per liter when the reaction is half complete.

TABLE VII

EFFECT OF TRIIODIDE

$$K_1 = (I_3^-)/(I_2)(I^-) = 6.85. \quad K_2 = (I_2Cl^-)/(I_2)(Cl^-) = 1.56. \quad (I_2, \text{tot.}) = 0.00248 \text{ } M.$$

Expt.	(I^-)	(Cl^-)	(I_2)	(I_3^-)	(I_2Cl^-)	<i>k</i>
A	0.0145	0.590	0.000239	0.00203	82%	0.000220 1.11
B	.0345	.570	.000103	.00228	92%	.000092 1.23
C	.0645	.540	.000056	.00238	96%	.000047 1.25

It is evident that in these experiments the increase in the velocity constant runs parallel to the increase in triiodide concentration. If only the triiodide reacts with cystine, one would expect to obtain in Expt. B a velocity constant of $92 \times 1.11/82 = 1.24$ and in Expt. C a velocity constant of $96 \times 1.11/82 = 1.29$. The velocity constants observed are 1.23 and 1.25 in Expts. B and C, respectively. And as the curve in Fig. 1 shows, in Expt. C the velocity constant is increasing with time even after 420 minutes.

From these experiments, one would conclude that if I_2 and I_2Cl^- react with cystine, they react at a much slower rate than does I_3^- . It should, however, be pointed out that in the buffer solutions little or no effect of iodide was detected; see Expts. A₁ and B₄, Table III. The increase in the velocity constant with time to be seen in the experiments of Fig. 1 was also observed in those experiments of Table V at hydrogen-ion concentrations above 0.2 *M*. The phenomenon appears whenever the product of hydrogen-ion and iodide concentrations exceeds 0.003. The authors do not know whether it is due to instability of the hydriodic acid, or to some other complication.

If the assumption is correct that the triiodide ion is the reactant in the oxidation of cystine by iodine, at least two factors enter into the effect upon the velocity constant of increase in the concentration of sodium chloride, shown in Table IV. In the first place, with constant iodide concentration and increasing chloride concentration, the fraction of the total iodine present as triiodide decreases. In the second place, there exists a primary salt effect upon the reaction between cystine and the triiodide ion.

The authors wish to express their gratitude to Mr. Robert McNeil, who granted the fellowship which made this work possible,

(15) Jones and Kaplan, *This Journal*, **50**, 1600, 1845 (1928).

(16) La Mer and Lewinsohn, *J. Phys. Chem.*, **38**, 171 (1934).

(17) Spivey and Dawson, *J. Chem. Soc.*, 1838 (1932).

Summary

1. In acid solution the oxidation of cystine by iodine to cysteic acid is practically quantitative. The rate-determining step in the oxidation is the reaction between a molecule of cystine and a molecule of iodine (or substances whose concentrations are proportional to their concentrations).

2. Over a wide range of hydrogen-ion concentration (P_{H} 1-5) the velocity constant remains almost constant.

3. In the range 0.01-0.06 M iodide, the ve-

locity constant increases with increase in iodide concentration. This effect, together with the absence of pronounced effect of the hydrogen ion, makes it appear probable that the triiodide ion is the reactant.

4. In those solutions where the product of hydrogen-ion and iodide concentrations exceeds 0.003, the velocity constant increases with time during an experiment. It is not known whether this is due to the instability of the hydriodic acid, or to some other complication.

PHILADELPHIA, PA.

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[CONTRIBUTION FROM THE CHEMISTRY LABORATORY OF THE UNIVERSITY OF NORTH CAROLINA]

The Ionization Constants of Certain Sulfur Derivatives of Phenylacetic Acid at 0 and 25°¹

BY H. D. CROCKFORD AND T. B. DOUGLAS

The purpose of the work given in this paper was to study the effect produced on the ionization constant by successively attaching, in an organic acid, oxygen atoms to an atom capable of assuming higher valences. To this end the ionization constants of phenylthioglycolic acid, phenylsulfoacetic acid and phenylsulfoxyacetic acid were determined conductimetrically at 0 and 25°. From the data have been calculated the heat of ionization and the free energy of ionization of the compounds.

Behaghel² determined the ionization constants of the first two acids at 25° using the simple dilution law formula. We have employed for our calculations the Onsager equation used by MacInnes and Shedlovsky³

$$\log k = \log \frac{\alpha^2 m}{1 - \alpha} - A \sqrt{c_1}$$

in which $\alpha = \Lambda/\Lambda_e$ and Λ_e is the equivalent conductance of a mole of completely dissociated electrolyte at the concentration m . C_1 is the ion concentration. The constant A has a value of 0.98 at 0 and 1.01 at 25°. Our results for the values determined by Behaghel differ from his in a manner that cannot be accounted for on the basis of the two different equations used in the calculations. We have therefore included these two data in this paper.

(1) From the thesis of Mr. Douglas presented to the Graduate Faculty in partial fulfillment of the requirements for the degree of Master of Science.

(2) Behaghel, *J. prakt. Chem.*, **114**, 287 (1926).

(3) MacInnes and Shedlovsky, *This Journal*, **54**, 1429 (1932); Shedlovsky, *ibid.*, **54**, 1411 (1932).

Experimental

Phenylthioglycolic Acid, $C_6H_5SCH_2COOH$.—This acid was prepared by the action of sodium thiophenylate on ethyl chloroacetate. After repeated crystallizations the acid was dried over phosphorus pentoxide for one and a half months, m. p. 63.0-63.5°.

Phenylsulfoxyacetic Acid, $C_6H_5SOCH_2COOH$.—Phenylthioglycolic acid was oxidized with 30% hydrogen peroxide and the product recrystallized from a mixture of ethyl acetate and benzene (1:3 by volume), m. p. 112.5-113°.

Phenylsulfonylacetic Acid, $C_6H_5SO_2CH_2COOH$.—The sodium salt of phenylthioglycolic acid was oxidized in alkaline solution with potassium permanganate at 0°. The acidified filtrate was extracted with ether and the acid recrystallized four times from benzene, m. p. 110.0-110.5°.

A Type 293-A General Radio Universal Bridge with a 1000-cycle oscillator was employed for the measurements. All measuring apparatus was properly calibrated. The conductance cell was frequently checked with potassium chloride solution. Temperatures were accurate to 0.02°. Due to the nature of the compounds studied the concentrations of the solutions involve an uncertainty of 0.1%.

Calculations and Results

Values for Λ_e as used in the Onsager equation were obtained from our values for the sodium salts of the acids used and the values of Λ given in the "International Critical Tables" for sodium chloride and hydrochloric acid. The usual method of approximation was employed. All experimental values were the average of at least four sets of conductivity data which showed a variation of less than 0.2%. Table I gives the values of the dissociation constants for the various concentrations of the three acids. Table II gives the average values of the constants, the free