[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, THE UNIVERSITY OF TEXAS]

The Decomposition of Urea in Aqueous Media

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RECEIVED APRIL 27, 1955

The reaction was studied at various urea concentrations ranging from 6 to 1200 millimolar. By employing the method of initial rates the reaction was shown to be first order with respect to urea. Data were collected at six temperatures in the presence and absence of acid and the frequency factors and energies of activation calculated. The reaction is not catalyzed by acid nor was any large change in rate observed with increasing ionic strength. Various mechanisms are discussed in the light of these findings and pertinent data from the literature are presented.

Introduction

The decomposition of urea in aqueous media at elevated temperatures has been previously studied by several investigators. Walker and Hambley[1] first called attention to the reversibility of Wöhler's classic synthesis. Fawsitt,[2,3] Werner,[4,5] Price,[6] and Warner[7] have studied the reaction in the presence of acids and bases. Krasilshchikov[8,9] and Moelwyn-Hughes[10] have calculated activation energies but the values differ by several kilocalories. Quite recently Laidler[11,12] has assumed the decomposition to be acid catalyzed. Pertinent work on the effect of isotopic substitution by Schmitt and Daniels[13] and a study of the decomposition in fatty acid solvents by Mukaiyama and Matsunaga[14] should also be mentioned.

The reverse reaction, the conversion of ammonium cyanate into urea, has been the subject of many kinetic investigations.[15] It has in fact been used as a sort of "test case" for various theories of solution kinetics.[16] Frost and Pearson[17] have recently presented an excellent discussion of the mechanism of this reaction.

Although the earlier workers[2–5] agreed that ammonium cyanate is an intermediate in the decomposition of urea, the numerical magnitudes of the rate constants reported and the various mechanisms postulated were quite different. Warner[7] has emphasized these ambiguities and has presented careful rate measurements at two temperatures over a wide *p*H range. He did not, however, report an activation energy for the reaction.

The present work was undertaken: to determine the entropy and energy of activation; to obtain additional information concerning the effects of various variables such as acid concentration, ionic strength, etc.; to attempt further to elucidate the mechanism; and to correlate the findings, if possible, with results obtained from enzyme studies.[18,19]

Experimental

Apparatus and Technique.—The reaction was carried out in sealed reaction vessels. The vessel was made from a 40 × 100 mm. test-tube, and contained a short length of smaller diameter (18 mm.) Pyrex tubing sealed to the bottom forming two coaxial cylinders. The test-tube was tapered down at the top and sealed to a length of 10 mm. Pyrex tubing. The resultant ampoule was employed in measurements designed to detect the presence or absence of acid catalysis. In use acid was added to the inner tube and the urea solution to the space between the coaxial cylinders. Several samples were placed in a specially constructed holder in a thermostated mineral oil-bath. The bath was well insulated and supplied with: a Variac controlled steady heater (500 watts) that was adjusted to almost compensate for heat loss; a 125-watt intermittent heater for final control; and a 1500-watt heater to bring the bath to the reaction temperature. The Aminco bimetallic thermoregulator maintained the temperature (as read by a thermometer calibrated by the Bureau of Standards) to about ±0.05°. Fifteen minutes was needed for temperature equilibration. After this time several ampoules, to be used as controls, were removed and the reaction quenched by plunging them into cold water. The remaining ampoules were simultaneously inverted *in situ* by a simple mechanical device and the acid and urea solution thoroughly mixed by repeated inversions. At the end of the reaction time the ampoules were rapidly removed and quenched as indicated above. For measurements with urea in water alone inner tubes were not needed in the reaction vessels.

Measurement showed that the volume change in the reaction mixtures at elevated temperatures could be neglected.

Analytical Method.—The reaction mixture was analyzed by isolating the ammonium ion on a cation exchanger, eluting, Nesslerizing the elutriate, and performing a photoelectric colorimetric analysis. The technique has been described previously.[20] The cyanate was quantitatively determined by acidifying the original elutriate from the ammonium determination whereupon the cyanate is rapidly and quantitatively converted to ammonium ion. The resultant ammonium ion is then determined as usual. A complete discussion of the method has been presented elsewhere.[21]

Reagents.—J. T. Baker analyzed C.P. urea, m.p. 133°, was used without further purification. All other reagents were of analytical reagent grade and conformed to A.C.S. specifications.

Results

It was felt that a reaction of this type might best be treated by the method of initial rates. Previous

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(1) J. Walker and F. J. Hambley, *J. Chem. Soc.* (*London*), **67**, 746 (1895).

(2) C. E. Fawsitt, *Z. physik. Chem.*, **41**, 601 (1902).

(3) G. J. Burrows and C. E. Fawsitt, *J. Chem. Soc.*, (*London*), **105**, 609 (1914).

(4) E. A. Werner, *ibid.*, **113**, 84 (1918).

(5) E. A. Werner, *ibid.*, **117**, 1078 (1920).

(6) T. W. Price, *ibid.*, **115**, 1254 (1919).

(7) R. C. Warner, *J. Biol. Chem.*, **142**, 709 (1942).

(8) A. I. Krasilshchikov, *J. Phys. Chem.* (*USSR*), **13**, 767 (1939); *C. A.*, **34**, 7706 (1940).

(9) A. I. Krasilshchikov, *ibid.*, **13**, 1429 (1939); *C. A.*, **35**, 371 (1941).

(10) E. A. Moelwyn-Hughes, "The Kinetics of Reactions in Solution," Second Edition, Oxford University Press, London, 1947, pp. 166, 98, 94, 71, 34.

(11) K. J. Laidler and J. P. Hoare, THIS JOURNAL, **72**, 2489 (1950).

(12) K. J. Laidler, "Chemical Kinetics," McGraw-Hill Book Co., Inc., New York, N. Y., 1950, p. 310.

(13) J. A. Schmitt and F. Daniels, THIS JOURNAL, **75**, 3564 (1953).

(14) T. Mukaiyama and T. Matsunaga, *ibid.*, **75**, 6209 (1953).

(15) A. A. Frost and R. G. Pearson, "Kinetics and Mechanism," John Wiley and Sons, Inc., New York, N. Y., p. 265.

(16) E. S. Amis, "Kinetics of Chemical Change in Solution," The Macmillan Co., New York, N. Y., 1949.

(17) Reference 16, pp. 257–265.

(18) G. B. Kistiakowsky and W. H. R. Shaw, THIS JOURNAL, **75**, 866 (1953).

(19) W. H. R. Shaw, *ibid.*, **76**, 2160 (1954).

(20) G. B. Kistiakowsky, P. C. Mangelsdorf, A. J. Rosenberg and W. H. R. Shaw, *ibid.*, **74**, 5015 (1952).

(21) W. H. R. Shaw and J. J. Bordeaux, *Anal. Chem.*, **27**, 138 (1955).

investigators had all employed an integrated rate expression; but it seemed reasonable to believe that, in the event of a significant amount of back reaction, more reliable constants could be obtained by the former method. The highly sensitive Nessler technique was ideally suited to this purpose. It permitted measurement of ammonium ion concentrations that corresponded to only a few per cent. conversion of urea to reaction products. To justify the basic assumption of the method of initial rates applied to this case (*i.e.*, that $\Delta u/\Delta t$ can be set equal to du/dt, where u is the urea concentration and t the time) a product–time curve, Fig. 1, was obtained as follows. (1) Six samples were thermostated. (2) After 15 minutes (see Experimental) three were removed and quenched. (3) The other samples were allowed to run 15 additional minutes. (4) All the samples were analyzed and the ammonium ion concentration found in the blank (step 2) was subtracted from that found in the sample (step 3). This result represented the ammonium ion concentration produced in a 15-min. reaction time at the bath temperature after thermal equilibration. (5) The process was repeated for several different reaction times and the results plotted. The straight line obtained indicated that under these conditions the method of intial rates could be used. The product-time curves in 0.05 *M* H_2SO_4 were straight for all temperatures and per cent. conversions studied. In water alone, however, the curves showed a decided departure from linearity. The effect was increasingly pronounced with increasing per cent. conversion. A detailed analysis of this phenomenon is presented in a subsequent paragraph.

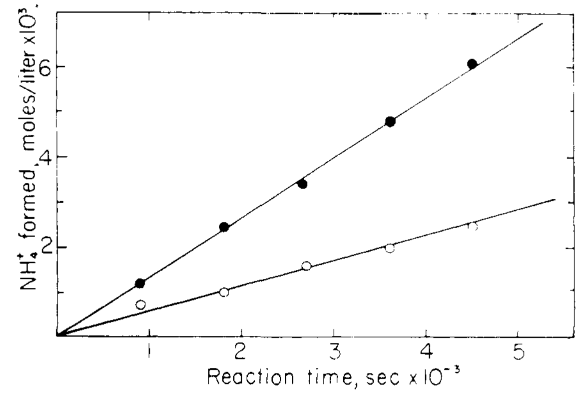


Fig. 1.—The product–time curves for the decomposition of urea in water (○) and in 0.05 *M* H_2SO_4 (●) at a temperature of 90.0°. The reaction mixtures were initially 60.0 m*M* in urea.

It was found possible to account quantitatively for all the reaction products in the absence of acid by the equation

$$u \xrightarrow{k_1} NH_4^+CNO^- \qquad (1)$$

The applicability of equation 1 was determined by analyzing for both cyanate and ammonium ion. In acid a rapid quantitative conversion of the cyanate ion to ammonium ion occurs.

$$CNO^- + 2H^+ + 2H_2O \longrightarrow NH_4^+ + H_2CO_3 \quad (2)$$

This reaction is complete at room temperature in sufficiently concentrated acid solution.[21] These findings are in complete agreement with those of Warner[7] and previous investigators.[2–6] They also account for the fact that the slope of the product (NH_4^+)–time curve for the reaction in acid is, within experimental error, twice that of the product–time curve for urea in water alone.

The effect of changing acid concentration was next examined (Fig. 2). This curve is very similar to that obtained by Fawsitt[2] with sulfuric and hydrochloric acids, and by Price[6] with nitric acid. The initial portion of the curve is easily explained.[21] If insufficient acid is present reaction (2) is not complete. If sufficient acid is present all the cyanate ion is quantitatively converted to ammonium ion. The plateau represents complete conversion and is consequently twice the intercept (see reaction (2)). The decrease at high acid concentration is in part due to analytical difficulties,[21] and most probably also to the existence of the following equilibrium.

$$u + H^+ \rightleftarrows uH^+ \qquad (3)$$

It is assumed that uH^+ cannot be directly decomposed into ammonium cyanate. Such a mechanism has been postulated previously.[2–7]

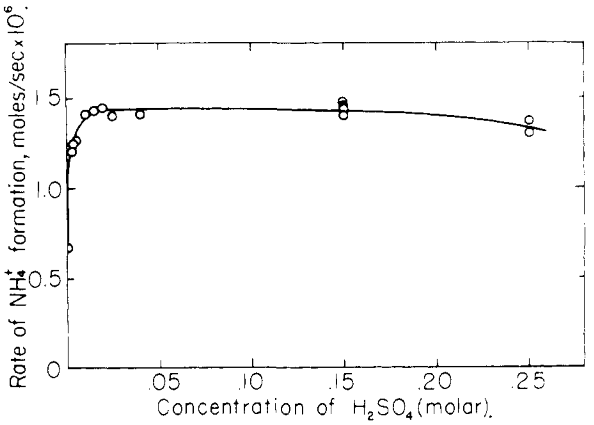


Fig. 2.—The rate of NH_4^+ production as a function of acid concentration.

The reaction was shown to be first order with respect to urea over a wide concentration range with the help of the following equations based on reaction (1).

$$-du/dt = k_1u \qquad (4)$$

Or in terms of the quantities actually measured

$$u_d/t = k_1\bar{u};\ k_1 = u_d/t\bar{u} \qquad (5)$$

where u_d is the urea decomposed in the reaction time t and k_1 is a first-order rate constant. The average urea concentration, $\bar{u}$, was employed in the calculation. It differed by only a few per cent. from the urea concentration initially present. For the reaction in 0.5 *M* sulfuric acid the urea decomposed was calculated by taking half of the net ammonium ion produced. The data obtained at various temperatures have been included in Table I and a least squares Arrhenius plot is exhibited in Fig. 3. The frequency factors and energies of activation so obtained together with those calculated from data reported by other investigators are included in Table II.

TABLE I

OBSERVED FIRST-ORDER RATE CONSTANTS FOR THE DECOMPOSITION OF UREA

Temp., °C. ± 0.05	Molarity × 10^3	Reaction time or range, sec.	$k_1 \times 10^5$, sec.$^{-1}$	No. of measurements
A. Medium: H_2O				
60.0	45.0–150.0	3600	0.0209 ± 0.0015[a]	5
70.0	30.0–149.8	3600	.0753 ± .005	6
80.0	5.95–148.9	3600	.332 ± .02	11
90.0	5.89–1192.6	900–4500	1.04 ± .10	41
90.0	58.0–60.0	3600	1.08 ± .07 (best value[b])	23
95.0	5.73–59.0	900–3600	2.18 ± .15[c]	12
100.0	5.62–115.4	3600	3.80 ± .27[d]	6
B. Medium: 0.050 *M* H_2SO_4				
60.0	45.0–150.0	3600	0.0207 ± 0.0015	10
70.0	29.9–149.7	3600	.0833 ± .006	6
80.0	29.8–148.8	3600	.385 ± .03	12
90.0	5.86–119.0	900–4500	1.20 ± .08	23
95.0	5.73–114.9	3600	2.20 ± .15	6
100.0	5.53–111.1	3600	4.15 ± .30	6

[a] The errors are standard deviations. [b] Based only on measurements involving small percentage decompositions. [c] Obtained by averaging three values fulfilling conditions outlined above. [d] Obtained by extrapolating to zero percentage decomposition.

TABLE II

ACTIVATION ENERGIES AND FREQUENCY FACTORS OBTAINED BY VARIOUS INVESTIGATORS

Medium	Frequency factor, sec.$^{-1}$	Activation energy, kcal.	Ref.
H_2O	4×10^{14}	32.4	This work
0.050 *M* H_2SO_4	6×10^{14}	32.7	This work
Composite[a]	5×10^{14}	32.7	This work
0.5 *N* HCl[b]	9×10^{9}	24.6	11, 12
HCl[c]	5×10^{13}	30.9	7
0.500 *N* HNO_3[d]	8×10^{13}	31.3	6
H_2O	......	31.3	3 *via* 10
H_2O	......	28.4	8

[a] Obtained by applying the least squares calculation to the data obtained in both acid and water under the assumption that the two sets were indistinguishable. [b] In the original work a frequency factor was reported based on the assumption that the reaction was acid catalyzed and second order. The value recorded in the table is that calculated for a first order uncatalyzed decomposition. [c] Calculated from Warner's data at *p*H 1.4. Rate constants at 100.0 and 66.0° were used in the computation. [d] Calculated by the method of least squares from data obtained by Price at four temperatures: 100, 89, 80 and 70°.

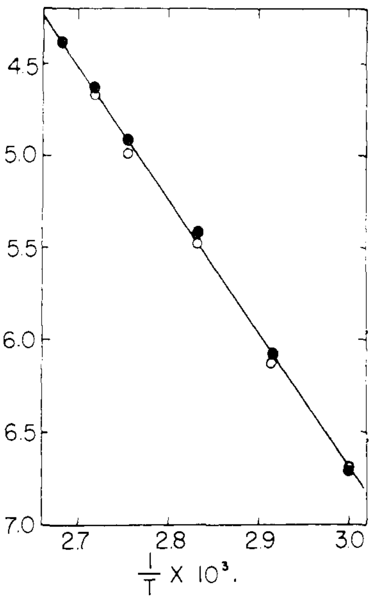


Fig. 3.—Arrhenius plot for the urea decomposition in water (○) and in 0.05 *M* H_2SO_4 (●).

Figure 4 depicts data obtained at 95° for various urea concentrations in both acid and water alone. A reaction time of one hour was used for all points except the one denoted by a square. Here a reaction time of 15 min. was employed. It is quite evident from the figure that the plot obtained for water alone has a decided curvature. Since more ammonium cyanate was produced (in the same time interval) at the higher urea concentration than at the lower ones, this curvature was tentatively attributed to the reverse reaction. Subsequent measurements at various time intervals were made. Since decreased reaction time corresponds to smaller per cent. conversion and smaller concentration of reaction products a decreased rate of the reverse reaction is to be expected. The results obtained were in accord with this argument and indicated that the reverse reaction is important when as little as 3% of the urea present initially has decomposed. A calculation based on the reported value[10] of the second-order rate constant at this temperature also indicated that this result was quite reasonable. It seems likely, therefore, that the drifting first-order constants obtained in previous work[2,3] can be explained on this basis, since an integrated first-order rate expression that did not make allowance for the reverse reaction was employed. The plot for the reaction in the presence of acid is straight since acid prevents the reverse reaction (equation 2). In this same connection, another point should be

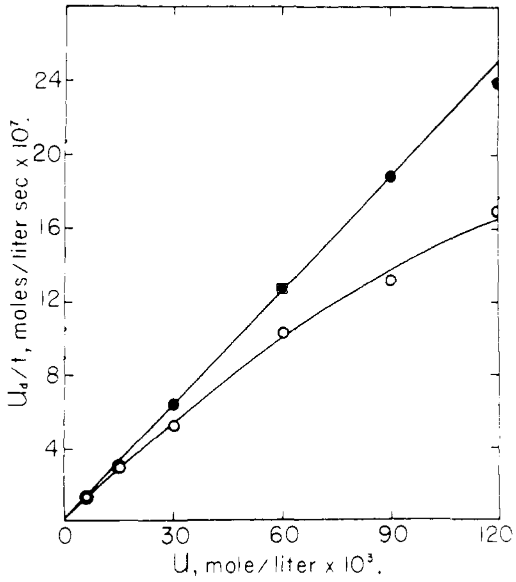


Fig. 4.—The rate of urea decomposition (u_d/t) at 95.0° as a function of urea concentration (u) in water (○ and □) and in 0.050 *M* H_2SO_4 (●). A reaction time of one hour was used for all points except the square. Here a reaction time of 15 min. was employed. Data indicate occurrence of reverse reaction.

mentioned. Although the results presented in Table I indicate that the rate constant in acid is, within the combined experimental uncertainties, the same as that in water, a slight trend is, nevertheless, evident. The constant in acid has a tendency to be somewhat higher than that in water. If it is assumed that in water alone a small amount of reverse reaction is occurring even at the low per cent. conversions employed in this study, then such a trend can be explained. Results obtained in media of varying ionic strength can also be interpreted in terms of this hypothesis (Table IV). These findings are analyzed in the following section.

Table III

Rate Constants for the Decomposition of Urea in Various Acid Solutions at 90.0°[a]

Acid	Molarity	Ionic strength	Av. urea concn., $M \times 10^3$	$k_1 \times 10^5$, sec.$^{-1}$
H_2SO_4	0.050	0.1[b]	5.86–119.0[c]	1.20 ± 0.08[c]
HNO_3	.100	.100	58.57	1.20
HNO_3	.100	.100	58.59	1.18
HNO_3	.100	.100	58.59	1.18
HCl	.100	.100	58.57	1.20
HCl	.100	.100	58.66	1.11
HCl	.100	.100	58.59	1.18
H_3PO_4	.050	.05[b]	58.57	1.19
H_3PO_4	.050	.05[b]	58.66	1.11
H_3PO_4	.050	.05[b]	58.60	1.16

[a] A reaction time of one hour was employed for all recorded values except the first. [b] These values are rough approximations since precise values of the ionization constants at this temperature were not available. [c] See Table I.

Table IV

Data Obtained at 90.0° in Media on Varying Ionic Strength

Added substance and concn.	Ionic strength	Av. urea concn., $M \times 10^3$	$k_1 \times 10^5$, sec.$^{-1}$
None	$1^a \times 10^{-3}$	58.0–60.0[b]	1.08 ± 0.07[b]
0.050 *M* H_3PO_4	0.05[c]	58.61	1.15
.100 *M* HCl	.100	58.61	1.16
.100 *M* HNO_3	.100	58.58	1.19
.050 *M* H_2SO_4	.1[c]	5.86–119.0[b]	1.20 ± 0.08[b]
.050 *M* $KHSO_4$	.1	58.61	1.14
.050 *M* K_2SO_4	.150	58.53	1.20
.050 *M* K_2SO_4	.150	58.70	1.05
.150 *M* KCl	.150	58.70	1.07
.150 *M* NaCl	.150	58.70	1.05
.150 *M* $NaNO_3$	.150	58.71	1.06
.050 *M* K_2SO_4 + .0250 *M* H_2SO_4	.2	58.52	1.24
.150 *M* $NaNO_3$ + .025 *M* H_2SO_4	.2	58.51	1.26
.150 *M* KCl + .025 *M* H_2SO_4	.2	58.49	1.28
.050 *M* K_2SO_4 + .050 *M* H_2SO_4	.2	58.61	1.16
.100 *M* K_2SO_4	.300	58.66	1.10
.167 *M* Na_2SO_4	.500	58.46	1.30
.333 *M* Na_2SO_4	1.100	58.50	1.27
.500 *M* Na_2SO_4	1.50	58.47	1.30
.666 *M* Na_2SO_4	2.00	58.50	1.27

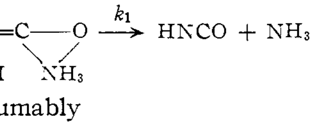
[a] This represents an approximate value of the average ionic strength of the ammonium cyanate produced in the reaction. [b] See Table I. [c] These ionic strength values are rough approximations since precise values of the ionization constants at this temperature were not available.

Experiments were also performed with different acids and are recorded in Table III. These findings indicate that the anions are without significant influence.

Discussion

The activation energy for the reaction in the presence of acid is, within the limit of experimental error, the same as that found for the reaction in water alone. This fact coupled with the preceding discussion (see Results) forces one to conclude that acid is not a catalyst[11,12] for the urea decomposition; but is instead, quite probably, an inhibitor.

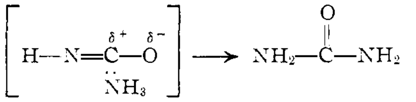
Two mechanisms for the decomposition of urea have previously been postulated, and enthusiastically defended by their authors. In a series of papers attempting to establish the structure of urea as I rather than the conventional diamide structure, Werner[4,5] presented the scheme


I (6)

and then presumably

$$HNCO + NH_3 \rightleftharpoons NH_4^+ + CNO^- \qquad (7)$$

Fawsitt[2,3] felt that this formulation was unnecessary and assumed instead a formulation identical with reaction (1). It is quite interesting to record that an entirely analogous discussion concerning the synthesis of urea is to be found in the literature.[15] The reverse of the Werner mechanism has been formulated by assuming equilibrium (7) followed by a combination of ammonia and cyanic acid *via* the scheme



$$\left[H{-}N{=}\overset{\delta+}{\underset{\ddot{N}H_3}{C}}{-}\overset{\delta-}{O} \right] \longrightarrow NH_2{-}\overset{\overset{\displaystyle O}{\|}}{C}{-}NH_2 \qquad (8)$$

II

The Werner structure I is essentially the same as the postulated reaction intermediate II.

Svirbely and Warner[22] have favored an ionic mechanism, the reverse of Reaction 1, and presented data at various ionic strengths that were in accord with this mechanism if a primary salt effect were postulated. It has been pointed out,[17] however, that these data are also in accord with the reverse of the Werner scheme (reactions (6) and (7)) if they are interpreted as a secondary salt effect on reaction (7).

It would appear that a study of the effect of added electrolytes on the decomposition of urea might be free from the ambiguities attending its synthesis. Thus if the reaction proceeded by the ionic mechanism the activated complex should be appreciably more polar than if the Werner mechanism were correct. The theoretical considerations of Kirkwood[23] and others[24] lead to the conclusion that an increased reaction rate with increased ionic strength is to be expected with reactions that give ionic products. For the decomposition of urea at 90.0° in water the theory relates k_μ, the rate con-

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[22] W. J. Svirbely and J. C. Warner, This Journal, **57**, 1883 (1935).

[23] J. G. Kirkwood, *Chem. Revs.*, **24**, 233 (1939).

[24] L. C. Bateman, M. G. Church, E. D. Hughes, C. K. Ingold and N. A. Taher, *J. Chem. Soc.*, (*London*), 979 (1940).

stant at finite ionic strength, to k_0, the rate constant at zero ionic strength, by the equation

$$\log k_\mu/k_0 = 2.06 \times 10^7 Z^2 d \qquad (9)$$

where Z is the charge born by the dipolar activated complex, d is the distance of charge separation and μ is the ionic strength. Assuming Z is approximately $^1/_2$, d is of the order of magnitude of the C–N bond distance (~1.4 Å.), this reduces to

$$\log k_\mu/k_0 = 0.0722\mu \qquad (10)$$

It is apparent from the equation that the effect will be relatively small in water at 90.0°. An inspection of our results obtained at varying ionic strengths (Table IV) reveals that because of experimental uncertainty it is not possible to make a clear-cut distinction between the ionic mechanism and the Werner mechanism from these data. The tendency for the rate in water alone to be somewhat lower than that in acid can be explained on two bases. First, if a small amount of reverse reaction were occurring even at the low per cent. conversions studied, added electrolyte would inhibit the reverse reaction and cause an apparent increase in the forward reaction. This phenomenon was most probably observed in the earlier work[2,4] done at relatively high per cent. conversions in water alone. A second explanation is that the ionic mechanism for the decomposition of urea is the correct one, and the rate in acid is faster because of the higher ionic strength of the acid solution. Fawsitt's observation[3] that the decomposition in water–alcohol mixtures is slower than in water alone can, perhaps, be interpreted as also favoring this point of view.

Pertinent thermodynamic data, an analysis of the entropy and energy of activation, and a discussion of the relevance of these findings to the mechanism of the urease-catalyzed hydrolysis of urea will be presented in a subsequent communication.

Acknowledgment.—The authors gratefully acknowledge the generous grant from Research Corporation of New York that made this study possible. They would also like to express their appreciation to Bernard Grushkin and Byron R. Lowrance for computational assistance.

AUSTIN, TEXAS

[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY OF THE UNIVERSITY OF MINNESOTA]

Equilibrium Constants of Exchange Reactions of Cystine with Glutathione and with Thioglycolic Acid Both in the Oxidized and Reduced State

BY I. M. KOLTHOFF, W. STRICKS AND R. C. KAPOOR

RECEIVED MARCH 23, 1955

Equilibrium constants have been determined of the following reactions: RSSR + GSSG(TSST) ⇄ 2RSSG(2RSST); RSSR + GSH(TSH) ⇄ RSSG(RSST) + RSH; RSSG(RSST) + GSH(TSH) ⇄ GSSG(TSST) + RSH and RSSR + 2GSH(2TSH) ⇄ GSSG(TSST) + 2RSH in which RSSR, GSSG and TSST denote the oxidized forms and RSH, GSH and TSH the reduced forms of cystine, glutathione and thioglycolic acid. The constants were found from solubility determinations of cystine in the various equilibrated reaction mixtures. The results were substantiated by some polarographic determinations. All the equilibrium constants were found of the order of one and unaffected by variations in the charge type of amino and carboxyl groups. From the equilibrium constants the oxidation potentials of the glutathione (GSH–GSSG) and thioglycolic acid (TSH–TSST) systems were calculated to be practically equal to that of the cystine–cysteine systems. The mixed disulfide RSST was found to be subject to a relatively rapid fission process in buffers of *p*H 5 to 7, while the other simple or mixed disulfides were stable under our experimental conditions.

Cysteine and thioglycolic acid have long been used as reducing agents for disulfide groups in proteins.[1] In recent years much importance is being attached to the sulfhydryl–disulfide interaction in the interpretation of phenomena observed in protein denaturation.[2–5]

A quantitative study is planned in this Laboratory of reactions involving low molecular weight thiols and disulfides on the one hand and native and denatured proteins on the other. In order to gain further insight into the characteristics of such reactions we have first studied interchange reactions between low molecular weight disulfides with formation of mixed disulfides and the reactions between disulfides and thiols. The equilibrium constant of the latter type of reaction allows the calculation of the oxidation potential of the particular systems. The results of these investigations are reported in this paper.

After Goddard and Michaelis[6] had shown that thioglycolic acid reacts with cystine with formation of cysteine Bersin and Steudel[7] made a quantitative study of the optical rotation of this system. They derived the equilibrium constant of the above reaction, assuming that dithiodiglycolic acid and cystine do not form a mixed disulfide. As shown in the present paper this assumption is entirely incorrect. Studies on reactions between simple and water-insoluble alkyl disulfides and mercaptans were reported by Gorin, *et al.*[8] In recent years mixed disulfides ($RSSR_1$) involving cystine and glutathione

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[1] See *e.g.*, F. G. Hopkins, *Biochem. J.*, **19**, 787 (1925); A. E. Mirsky and M. L. Anson, *J. Gen. Physiol.*, **18**, 307 (1935); *ibid.*, **19**, 427 (1936); F. H. Haurowitz and M. Kennedy, *Proc. Fed. Am. Soc. Exp. Biol.*, **II**, 227 (1952).

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[5] V. D. Hospelhorn and E. V. Jensen, *ibid.*, **76**, 2830 (1954).

[6] D. R. Goddard and L. Michaelis, *J. Biol. Chem.*, **106**, 605 (1934).

[7] T. Bersin and J. Steudel, *Ber.*, **71B**, 1015 (1938).

[8] G. Gorin, G. Dougherty and A. V. Tobolsky, THIS JOURNAL, **71**, 2551 (1949).