cients found in this reaction, and that the method of initial rates must be used unless it is desired to go through the somewhat elaborate treatment of this inhibition.

Summary

1. The inhibition of the urease-catalyzed hydrolysis of urea by the product ammonium ions has been investigated, by the addition of various amounts of the ions and the measurement of initial rates. The inhibition is found to be of the non-competitive type.

2. The complete rate law, taking account of the inhibition by products, has been integrated, and it is found that a satisfactorily constant first-order coefficient is obtained. The inhibition is therefore adequate to account for the drifting first-order constants that have generally been found.

WASHINGTON, D. C. RECEIVED AUGUST 8, 1949

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, THE CATHOLIC UNIVERSITY OF AMERICA]

The Molecular Kinetics of the Urea–Urease System. III. Heats and Entropies of Complex Formation and Reaction[1]

BY K. J. LAIDLER AND J. P. HOARE

Introduction

In a previous paper[1a] (Part I) it was suggested that the kinetics of the urease-catalyzed hydrolysis of urea could best be interpreted on the basis of a model involving the formation of a complex consisting of the enzyme, the substrate, and a water molecule. The mechanism may be represented as

$$\text{Urea} + \text{urease} + H_2O \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} \text{urea–urease–}H_2O$$

$$\text{Urea–urease–}H_2O \xrightarrow{k_1} \text{urease} + \text{products}$$

where k_1, k_{-1} and k_0 are the rate constants. The resulting rate equation is

$$v = \frac{k_0 K_1 c_s c_e}{(1 + K_1 c_s)(1 + K_2 c_s)} \qquad (1)$$

where c_s and c_e are the initial concentrations of substrate and enzyme, and K_1 and K_2 are constants. If the intermediate complex can be assumed to be in equilibrium with the reactants, *i. e.*, if $k_2 \ll k_{-1}$, K_1 is the equilibrium constant k_1/k_{-1}, while K_2 is the analogous constant for the process

$$\text{Urea–urease–}H_2O + \text{urea} \rightleftarrows \text{urea–urease–urea} + H_2O$$

It was found that the best agreement with the data was obtained with $K_1 = K_2 = K$, so that the rate equation reduces to

$$v = \frac{k_0 K c_s c_e}{(1 + K c_s)^2} \qquad (2)$$

In the present paper kinetic data at four temperatures are analyzed to give, on the basis of the above mechanism, heats and entropies of reaction corresponding to the formation of the enzyme-substrate complex, and heats and entropies of activation for the decomposition of the complex. This work was undertaken with the object of obtaining thermodynamic and pseudo-thermodynamic data which may help to throw some light on the mechanism of enzyme action.

(1) Abstracted from a dissertation submitted by J. P. Hoare in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Chemistry at the Catholic University of America, June, 1949.

(1a) K. J. Laidler and J. P. Hoare, THIS JOURNAL, **71**, 2699 (1949).

Experimental

Runs were made according to the procedure described in Parts I and II[2] at the temperatures 20, 30, 40 and 50°; at the latter temperature the rate of deactivation of the urease is measurable, but it was verified that this was not sufficiently great to affect the over-all rate of hydrolysis to a significant extent. Duplicate runs were carried out in phosphate buffers at *p*H 6.6 and 6.2. In addition to the enzyme-catalyzed reaction, the acid-catalyzed hydrolysis was investigated at the temperatures 62, 70, 85 and 100°, using 0.5 *N* hydrochloric acid.

Results and Discussion

The Acid-Catalyzed Reaction.—The first-order rate constants k for the acid-catalyzed reaction were calculated using the ordinary rate equation $k = (1/t) \ln [a/(a - x)]$, where a is the initial substrate concentration and x is that after time t. The constancy of the k's at a given temperature as shown in Table I indicates that the

TABLE I

TYPICAL FIRST-ORDER RATE CONSTANTS FOR THE ACID-CATALYZED REACTION (T = 70°)

Time, sec.	$k \times 10^5$, sec.$^{-1}$
75,300	0.144
89,700	.146
99,000	.123
184,800	.142
259,200	.133
323,100	.147

TABLE II

RATE CONSTANTS FOR THE ACID-CATALYZED REACTION

Temp., °C.	$k \times 10^7$, sec.$^{-1}$
62.0	7.38
70.0	13.9
85.0	65.2
100.0	359.0

(2) J. P. Hoare and K. J. Laidler, *ibid.*, **72**, 2487 (1950).

reaction is of the first order with respect to urea. Mean rate constants for the four temperatures investigated are collected in Table II, and in Fig. 1 the Arrhenius plot of these values is shown, the resulting activation energy being 24.6 kcal.

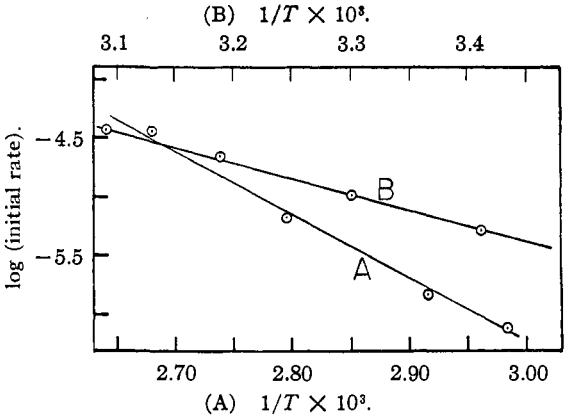

Fig. 1.—Arrhenius plots for the acid-catalyzed urea hydrolysis (A, activation energy = 24.6 kcal.) and the urease-catalyzed hydrolysis (B, pH 6.6, urea concentration 0.498×10^{-2} mole liter^{-1}, activation energy 12.5).

The rate of the acid-catalyzed reaction may be written as

$$v = k_1(\text{urea}) = k_2(H_3O^+)(\text{urea})$$

where k_1 is the first-order constant and k_2 a second-order constant. The constant k_2 was calculated and found to correspond to a frequency factor A of 1.80×10^{10} sec.$^{-1}$ for the reaction. According to the theory of absolute reaction rates[3] the frequency factor is given by

$$A = e^{kT/h}\, e^{\Delta S^*/R} \qquad (3)$$

where k is Boltzmann's constant, h is Planck's constant, T the absolute temperature, R the gas constant per mole, and ΔS^* the entropy of activation. In this way a value of -14.0 e. u. was calculated for the ΔS^* of the acid-catalyzed reaction.

The Urease-Catalyzed Reaction.—The rate of the enzyme-catalyzed reaction was determined by measurement of the initial slope of a plot of the amount of ammonia liberated as a function of the time. Rates were measured at four temperatures, and the activation energy obtained by plotting the logarithm of the rate against $1/T$, the procedure being repeated over a range of initial urea concentrations. The rates corresponding to a series of concentrations and temperatures, and a pH of 6.2, are given in Table III; the concentration of enzyme used was not necessarily the same for the various urea concentrations, but was the same for the four temperatures at each urea concentration. The activation energies for pH's 6.2 and 6.6 are shown in Table IV, and a typical Arrhenius plot is included in Fig. 1. The activation energies are observed to decrease with an increase in urea concentration.

(3) H. Eyring, *J. Chem. Phys.*, **3**, 107 (1935); *cf.* S. Glasstone, K. J. Laidler and H. Eyring, "The Theory of Rate Processes," McGraw-Hill Book Co., Inc., New York, N. Y., 1941, p. 199.

TABLE III

INITIAL RATES OF THE ENZYME-CATALYZED REACTION AT VARIOUS UREA CONCENTRATIONS AND TEMPERATURES (pH 6.2)

Initial urea concn., (mole liter^{-1}) $\times 10^2$	Rate (mole liter^{-1} sec.$^{-1}$) $\times 10^8$ 20°	30°	40.5°	50.5°
0.498	0.0595	0.121	0.260	0.428
2.49	0.167	0.297	0.508	0.865
7.48	2.16	4.22	6.40	9.92
14.96	4.84	7.34	10.62	15.55
29.91	7.34	10.30	14.69	20.87
74.78	6.07	8.43	12.70	16.36
149.6	4.08	5.63	7.14	10.11

TABLE IV

ENERGIES OF ACTIVATION FOR THE ENZYME-CATALYZED REACTION

Initial urea concn., (mole liter^{-1}) $\times 10^2$	Energy of activation, kcal. pH 6.2	pH 6.6
0.498	12.4	12.5
1.994	..	10.2
2.493	10.2	..
7.478	8.2	7.6
14.96	7.2	7.5
29.91	6.7	6.1
74.78	6.4	..
149.6	5.9	..

In the rate equation (2) it is seen that at low substrate concentrations, when $Kc_s \ll 1$, the rate is proportional to k_0K, while at high concentrations, when $Kc_s \gg 1$, the rate is proportional to k_0/K. The equilibrium constant K may be expressed as

$$K = e^{\Delta S/R}\, e^{-\Delta H/RT} \qquad (4)$$

where ΔS and ΔH are the entropy and heat of reaction corresponding to the formation of the intermediate complex. According to the theory of absolute reaction rates the rate constant k_0 is given by

$$k_0 = (kT/h)e^{\Delta S_0^*/R}e^{-\Delta H_0^*/RT} \qquad (5)$$

where ΔS_0^* and ΔH_0^* are the entropy and heat of activation for the decomposition of the intermediate complex. The rate at low urea concentrations may thus be expressed as

$$v_{\text{low}} = c_e c_s\, (kT/h)e^{(\Delta S_0^* + \Delta S)/R}e^{-(\Delta H_0^* + \Delta H)/RT} \qquad (6)$$

$$= c_e c_s\, (kT/h)e^{\Delta S^*/R}e^{-\Delta H^*/RT} \qquad (7)$$

where ΔS^* and ΔH^* are defined by

$$\Delta S^* = \Delta S_0^* + \Delta S \qquad (8)$$

$$\Delta H^* = \Delta H_0^* + \Delta H \qquad (9)$$

At high urea concentrations the rate is given by

$$v_{\text{high}} = \frac{c_e}{c_s}\frac{kT}{h}e^{(\Delta S_0^* - \Delta S)/R}e^{-(\Delta H_0^* - \Delta H)/RT} \qquad (10)$$

Two methods were used to analyze the experimental results obtained. The first involved determining the energies of activation at very low and very high urea concentrations. From the

rate *vs.* concentration curves at four different temperatures the initial slopes were obtained and the logarithms of these plotted against $1/T$; the activation energy so obtained is $\Delta H_{low}^* = \Delta H^* = \Delta H_0^* + \Delta H$ (*cf*, eq. (9)). If the reciprocal of the urea concentration is plotted against the rate at high urea concentrations, and the resulting slopes plotted against $1/T$, the corresponding heat of activation $\Delta H_{high}^* = \Delta H_0^* - \Delta H$, as is evident from eq. (10). In the light of these expressions for ΔH_{low}^* and ΔH_{high}^* it follows that

$$\Delta H_0^* = {}^1/_2(\Delta H_{low}^* + \Delta H_{high}^*) \qquad (11)$$

and

$$\Delta H = {}^1/_2(\Delta H_{low}^* - \Delta H_{high}^*) \qquad (12)$$

Application of this procedure to the data gave 12.5 kcal. for ΔH_{low}^* and 6.0 kcal. for ΔH_{high}^*, and these values give rise to $\Delta H = 3.25$ kcal. and $\Delta H_0^* = 9.25$ kcal. Since this method involves the measurement of initial slopes, an accuracy of better than 0.5 kcal. is not to be expected.

The second method, which is less reliable and is used as a rough check only, involved analyzing the individual rate-concentration curves for k_0 and K at the four temperatures investigated; this required a trial-and-error procedure since eq. (2) is not amenable to a least-square treatment. ΔH and ΔH_0^* are then obtained from the temperature variations of K and k_0, respectively. The values so obtained are $\Delta H = 4.8$ kcal. and $\Delta H_0^* = 8.0$ kcal. The discrepancy between the two sets of values is to be expected in view of the difficulty involved in carrying out the analysis of the experimental data. There is no doubt, however, that ΔH is a positive quantity, *i. e.*, the enzyme-substrate complex has a negative heat of formation; the significance of this will be considered later.

The value of ΔS can be obtained from K and ΔH, using eq. (4); the resulting value at *p*H 6.6 is 14.9 e. u. For the determinations of ΔS^* and ΔS_0^* the absolute molarity of the urease solution is required. The activity of the solution was determined according to the procedure described in Part I, and the molarity calculated on the basis of Sumner's result[4] that under the standard conditions employed 1 g. of pure urease produces 133,000 mg. of ammonia nitrogen; a molecular weight of 483,000 was assumed.[5] The first-order rate constant k^1, equal to $k_0 K c_e$, is converted to a second-order constant equal to $k_0 K$ by dividing by the urease concentration c_e. The entropy associated with $k_0 K$ is ΔS^*; k^1 was 2.77×10^{-4} sec.$^{-1}$ at 300° K. at *p*H 6.2, and the activation energy of 12.5 kcal. leads to a value of 6.9 e. u. for ΔS^*. The entropy of activation ΔS_0^* for the decomposition of the complex is thus −6.4 e. u.

The values for the heats and entropies at the two *p*H values are summarized in Table V; the figures quoted were obtained by the first procedure. The schematic drawings of Figs. 2 and 3

(4) J. B. Sumner, *J. Biol. Chem.*, **69**, 435 (1926).

(5) J. B. Sumner, N. Gralen and I. B. Eriksson-Quensel, *ibid.*, **125**, 37 (1938).

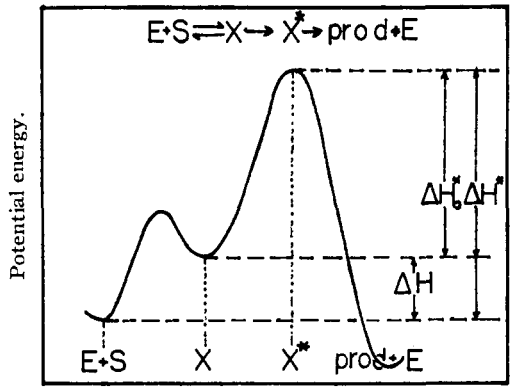

Fig. 2.—Schematic representation of the variation of the potential energy with the extent of reaction: X represents the intermediate complex and X* the activated complex.

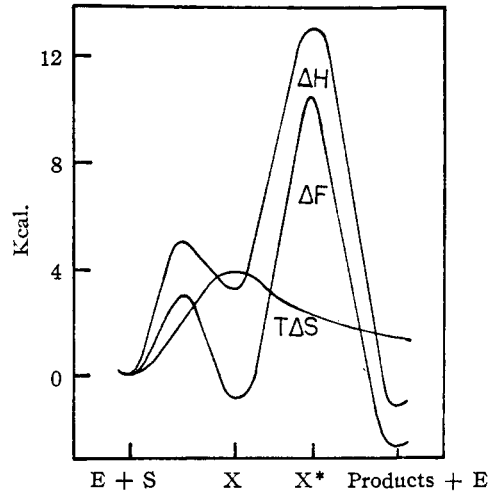

Fig. 3.—Schematic representation of the variation of *H*, *F* and *TS* with the extent of reaction.

represent the relative magnitudes of the quantities. Kistiakowsky and Lumry[6] have given −12.5 e. u. for the entropy change when the stable complex becomes the activated complex; this value differs from our ΔS_0^* values partly as a result of the different models used in interpreting the results.

TABLE V

HEATS, ENTROPIES AND FREE ENERGIES (T = 300°K.)

	*p*H 6.2	*p*H 6.6
ΔH^*, kcal.	12.5	12.5
ΔH_0^*, kcal.	9.25	9.25
ΔH, kcal.	3.25	3.25
ΔS^*, e. u.	6.9	7.5
ΔS_0^*, e. u.	−6.4	−7.5
ΔS, e. u.	13.3	14.9
ΔF^*, kcal.	10.5	10.3
ΔF_0^*, kcal.	11.1	11.2
ΔF, kcal.	−0.65	−0.98

(6) G. B. Kistiakowsky and R. Lumry, THIS JOURNAL, **71**, 2006 (1949).

Additional Evidence as to the Validity of the Mechanism Proposed.—The enzyme model proposed, involving the assumption that a urea molecule may displace a water molecule from a site normally reserved for it, was put forward to account for the falling-off of the rate at high urea concentrations. Since it had been suggested that this falling-off might be explicable as due simply to reduction in the activity of the water at high urea concentrations, this possibility has been investigated directly by freezing-point determinations on buffered urea solutions covering the range of concentrations used in the kinetic work. The freezing points obtained, relative to zero urea concentration, are given in Table VI, which includes also the activity of the water calculated from the freezing point lowering. It is to be noted that the activity of the water decreases by only 0.2% over the entire range, whereas at the corresponding urea concentration the rate is reduced by about 15% of the maximum value (*cf.* Fig. 2 of Part I). The decrease in the activity of the water is therefore much too small to explain the effect, and it seems necessary to postulate, as was done in Part I, specific and competitive adsorption of urea and water molecules on neighboring sites on the enzyme molecule.

TABLE VI

ACTIVITY OF WATER IN UREA SOLUTIONS

Urea concn., (mole liter^{-1}) $\times 10^2$	Water concn., mole liter^{-1}	T_f, °C.	Water activity, relative
0.00	55.27	0.000	1.0000
9.97	55.00	− .135	0.9994
12.45	54.98	− .185	.9992
14.96	54.87	− .235	.9990
19.94	54.71	− .330	.9968
29.91	54.29	− .450	.9981

General Discussion

The Entropies of Activation.—The entropy of activation for the acid-catalyzed reaction is −14.0 e. u. and this is of the same order of magnitude as those for a number of similar acid-catalyzed reactions, some values for which are included in Table VII. However, ΔS^* for the urease-catalyzed reaction is positive, and positive values have also been obtained by J. A. V. Butler[7] for a reaction catalyzed by trypsin, and by E. J. Casey and K. J. Laidler[8] for the pepsin-catalyzed hydrolysis of carbobenzoxy-1-glutamyl-*l*-tyrosine and of its ethyl ester; the values are included in Table VII. These facts indicate that the catalytic action of these hydrolytic enzymes is related both to a reduction in activation energy and to an increase in entropy of activation. On the other hand, the ΔS^* values for two reactions catalyzed by chymotrypsin[9] are found to be highly negative, and the ΔH^* values correspondingly low. The ΔS^* and ΔH^* values listed in Table VII appear to be the only ones published for enzyme-catalyzed reactions.

TABLE VII

HEATS AND ENTROPIES OF ACTIVATION FOR VARIOUS HYDROLYSIS REACTIONS

Substrate	Catalyst	ΔH^*, kcal. per mole	ΔS^*, e. u.	Ref.
Acetamide	H_3O^+	22.8	−14.9	*a*
Acetylglycine	H_3O^+	25.4	−15.6	*b*
Benzoylglycine	H_3O^+	25.2	−18.6	*b*
Urea	H_3O^+	24.5	−14.0	*c*
Urea	Urease	12.5	7.5	*c*
Chymotrypsinogen	Trypsin	16.3	8.5	*d*
Carbobenzoxy-1-glutamyl-1-tyrosine ethyl ester	Pepsin	23.1	16.1	*e*
Carbobenzoxy-1-glutamyl-1-tyrosine	Pepsin	20.2	4.6	*e*
Benzoyl-1-tyrosinamide	Chymotrypsin	3.1	−43.0	*f*
Benzoyl-1-tyrosine ethyl ester	Chymotrypsin	0.2	−38.5	*f*

[a] Mechan, Thesis, Liverpool, 1923; H. von Euler and Rudberg, *Z. anorg. Chem.*, **127**, 244 (1923). [b] Escolme and W. C. McC. Lewis, *Trans. Faraday Soc.*, **23**, 651 (1927). [c] Present work. [d] J. A. V. Butler, THIS JOURNAL, **63**, 2970 (1941); Butler's other values probably do not correspond to ΔH^* and ΔS^* (*cf.* ref. (8)). [e] E. J. Casey and K. J. Laidler, *ibid.*, **72**, 2159 (1950). [f] S. Kaufman, H. Neurath and G. W. Schwert, *J. Biol. Chem.*, **177**, 793 (1949).

To explain the positive entropies of activation found for some of these enzyme systems, and on the basis of additional evidence to be discussed in the next section, it is suggested that in the activated complex the enzyme molecule has undergone a change in structure associated with an increase in entropy.

The Equilibrium Constant for Complex Formation.—The results have been seen to indicate that the enzyme-substrate complex is formed endothermically from the enzyme and substrate, *i. e.*, that ΔH is positive, as is represented schematically in Fig. 2. This behavior is the opposite to that in the case of heterogeneous gas reactions, where the adsorption complexes are formed exothermically from the reactants.

The different behavior found in the urea–urease system and the trypsin[7] and pepsin[8] systems may be ascribed to two causes. In the first place, adsorption from solution is in reality an exchange reaction, a molecule of substrate in this case replacing one or more molecules of water. The process is therefore not necessarily accompanied by a loss of entropy: in fact if, as is probable, the urea has a highly orienting influence on neighboring water molecules there may be a gain of entropy and a corresponding gain of enthalpy. Klotz and Urquhart[10] have in fact found positive entropies in the formation of protein complexes.

In the second place, the entropy increase may be

(7) J. A. V. Butler, THIS JOURNAL, **63**, 2970 (1941).

(8) E. J. Casey and K. J. Laidler, *ibid.*, **72**, 2159 (1950).

(9) S. Kaufman, H. Neurath and G. W. Schwert, *J. Biol. Chem.*, **177**, 793 (1949).

(10) I. M. Klotz and J. M. Urquhart, THIS JOURNAL, **71**, 847 (1949).

due in part to an actual change in the structure of the enzyme on complex formation, evidence in favor of which was adduced above on the basis of the ΔS^* values. It is known that the deactivation of proteins is accompanied by a considerable increase in entropy, and it is suggested that the change in enzyme structure indicated by the present results may be somewhat of the same character as an enzyme deactivation, but proceeding in a reversible manner. Johnson, Eyring and their collaborators[11] have found that certain inhibitors of bacterial luminescence (belonging to their Type II) bring about the conversion of active enzymes into denatured forms, and a similar process may occur when the substrate forms a complex. Possibly the substrate combines with the enzyme at a center which is only exposed when the enzyme undergoes such a structural change.

The Variation of the Activation Energy with the Temperature.—A decrease of the activation energy with increasing temperature has been found in a number of biological experiments, and in particular Sizer has obtained this result with the urea–urease reaction. It has frequently been supposed that the activation energy changes sharply at a certain critical temperature. Sizer[12] has maintained that for the urea hydrolysis the activation energy is 11.7 kcal. below 23° and 8.7 kcal. above that temperature, but Kistiakowsky and Lumry[6] have recently shown by a statistical analysis that the data do not justify the conclusion that there is such a sudden change.

Any model involving the formation of a stable intermediate complex between the reactants requires that in general the activation energy will decrease with increasing temperature. This may be seen with reference to the schematic potential-energy curve represented in Fig. 2. At low temperatures the system will be largely in the state represented by $E + S$, and the activation energy will be approximately ΔH^*; at higher temperatures the system will be to a greater extent in the upper state X, and the activation energy will approach the lower value ΔH_0^*. The same result is also given if X is at a lower heat level than $E + S$; the system will then be in the state X at lower temperatures and in $E + S$ at higher ones, so that the energy of activation again decreases with increasing temperature.

The enzyme model that we have proposed, and the values we have obtained for the heats and entropies, are consistent in a general way with Sizer's experimental results, but not with his conclusion that there is a critical temperature separating two regions of higher and lower activation energies. Our results predict that log (rate) varies with $1/T$ in the manner shown in Fig. 4, the activation energies being indicated in the figure. This predicted variation in activation energy with temperature is, however, not sufficiently great to be detectable directly in our experiments, the available temperature range being much narrower than represented in Fig. 4. It is nevertheless of interest to note that Sizer's two energies (11.7 and 8.7 kcal.) are not far from the limiting values (12.5 and 6.0 kcal.) predicted by the present work.

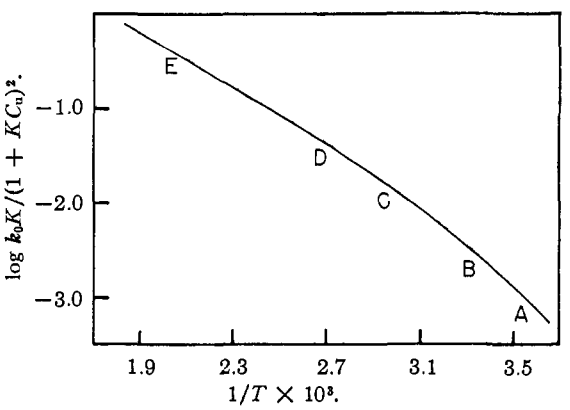

Fig. 4.—Theoretical Arrhenius plot for the urease-catalyzed reaction, based on the analysis of the present data in terms of the enzyme model proposed. The following values corresponding to the points indicated illustrate the fall of E with increasing temperature: A, 17°, 11.43 kcal.; B, 50°, 9.33 kcal.; 83°, 8.01 kcal.; D, 98°, 7.62 kcal.; E, 125°, 6.60 kcal.

Inhibition by Phosphate Ions.—Since the experimental work described in this paper was completed, K. M. Harmon and C. Niemann[13] have shown that urease is competitively inhibited by phosphate ions, present in the buffer system. This means, according to our mechanism, that strictly speaking either eq. (11) or eq. (13) of Part II[2] is applicable, rather than eq. (2) of the present paper.

The effect of this is to bring about a slight modification in the significance of ΔH^*, ΔS^*, ΔH_0^* and ΔS_0^*, which now apply to the inhibited reaction, but not of ΔH and ΔS, which are directly related to the equilibrium constant K. If a non-inhibiting buffer had been used, ΔS^* and ΔS_0^* would presumably be somewhat more positive than obtained here, while ΔS would be identical. The general conclusions reached in the preceding sections regarding entropy changes are therefore unaffected by the inhibition.

The conclusions regarding the variation of activation energy with temperature are also unaffected by the fact that there is inhibition, since this variation depends upon the magnitude of ΔH. Even in a completely uninhibited system a variation of E with T is therefore to be expected, although its direct detection would be difficult. In inhibited systems there is the additional reason for the variation that Kistiakowsky and Lumry have recently elucidated.

(11) F. H. Johnson, H. Eyring and R. W. Williams, *J. Cell. Comp. Physiol.*, **20**, 247 (1942); Johnson, Eyring, R. Steblay, H. Chaplin, C. Huber and G. Gherardi, *J. Gen. Physiol.*, **28**, 463 (1945).

(12) I. W. Sizer, *ibid.*, **22**, 719 (1939); *J. Biol. Chem.*, **132**, 209 (1940); I. W. Sizer and A. A. Tytell, *ibid.*, **138**, 681 (1941).

(13) K. M. Harmon and C. Niemann, *ibid.*, **177**, 601 (1949).

Summary

1. The acid-catalyzed hydrolysis of urea has been investigated, using 0.5 *N* hydrochloric acid in aqueous solution. The activation energy was 24.6 kcal. and the entropy of activation −14.0 e. u.

2. The urease-catalyzed hydrolysis of urea was investigated in phosphate-buffered solutions over a range of urea concentrations and temperatures. At low urea concentrations the activation energy was 12.5 kcal. and the entropy of activation 7.5 e. u. The activation energy decreases markedly with increasing urea concentration.

3. The results are interpreted quantitatively on the basis of an enzyme model involving the formation of a urea–urease–water complex, the urease being assumed to displace the water reversibly at high concentrations. The heat and the entropy of reaction for complex formation are 3.25 kcal. and 14.9 e. u. at *p*H 6.6, while the heat and the entropy of activation for complex decomposition into the products of reaction are 9.25 kcal. and −7.0 e. u.

4. It is suggested, in order to account for the positive value of the entropy of formation of the complex, and the increase in entropy in forming the activated state from the initial reactants, that a reversible structural change in the enzyme occurs, the molecule opening out, as in a deactivation, during complex formation.

5. The results are shown to be consistent with a decrease in activation energy with increasing temperature, the change being a gradual one and not a sharp one as had been suggested on the basis of certain previous experiments.

WASHINGTON, D. C. RECEIVED JUNE 6, 1949

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, THE STATE UNIVERSITY OF IOWA]

The Action of Bromine Vapor on Solid Aromatic Compounds[1]

BY ROBERT E. BUCKLES, EUGENE A. HAUSMAN AND NORRIS G. WHEELER

The reaction of solid aryl olefins with bromine vapor has been reported[2] as a method of synthesis of the corresponding olefin dibromides. It has been found in the present investigation that the action of bromine vapor on solid aromatic compounds leads to results similar to those expected from bromination in solution by an ionic mechanism.[3] Not only was the bromine often added to the double bond of an aryl olefin, but also bromination in the *p*-positions of unsubstituted phenyl groups took place. Aromatic compounds with no olefinic double bonds also underwent bromination by bromine vapor. The results are given in Table I.

A number of compounds with more highly substituted double bonds did not add bromine but underwent substitution of bromine for hydrogen in the *p*-positions of the phenyl groups and on the double bonds. Especially interesting is the case of the tetrakis-(*p*-bromophenyl)-ethylene obtained in the bromination of tetraphenylethylene. This tetrabromide exhibited a great affinity for bromine and adsorbed a good deal of bromine to give a powdery, green solid. This solid lost bromine on standing and regenerated the tetrabromide. The amount of bromine adsorbed depended qualitatively on the concentration of the bromine vapor. This behavior is reminiscent of the reactions of halogens with highly arylated ethylenes having groups in the *p*-positions capable of supplying electrons to the aromatic rings, *i. e.*, the so-called positive ethylenes.[4]

In most cases the aromatic compounds remained in the solid state throughout the reactions with bromine vapor. At the end of the reactions more or less sticky solid products were obtained. In a few experiments so much excess bromine was taken up that the products were partially dissolved in it. The reaction with bromine evidently took place in an adsorbed phase on the surface of the crystals, in a film of solution formed by the aromatic compound dissolved in liquid bromine, or in a combination of both. In either event the reaction must be similar to the ionic reactions of bromine in solution.[3] Both the addition of bromine to the double bond and the aromatic bromination are best explained by ionic mechanisms in which the double bond, the aromatic system, or a combination of the two exhibit basic character and complex with the bromine to form intermediates of the type, $ABr^{+}Br^{-}$. Such complexes have recently been studied by Benesi and Hildebrand[5] in the case of iodine and aromatic compounds. It would be expected that the more complex electronic systems of highly arylated double bonds might associate with more than one halogen molecule as has been reported by Witzinger[4b] in the case of the positive ethylenes. Such an ionic intermediate could then by the addition of bromide ion give olefin dibromide, by the loss of hydrogen bromide give a brominated

(1) Abstracted in part from two theses presented to the Graduate College of the State University of Iowa by Eugene A. Hausman and by Norris G. Wheeler in partial fulfillment of the requirements of the M.S. degree.

(2) (a) Baeyer, *Ber.*, **13**, 2254 (1880); (b) Elbs and Bauer, *J. prakt. Chem.*, [2] **34**, 243 (1886); (c) Sudborough and Thompson, *J. Chem. Soc.*, **83**, 666 (1903); (d) Wohlring, *Ber.*, **47**, 108 (1914).

(3) Price, "Reactions at Carbon-Carbon Double Bonds," Interscience Publishers, Inc., New York, N. Y., 1946, Chap. 2.

(4) (a) Witzinger, *J. prakt. Chem.*, [2] **154**, 1 (1939); (b) Witzinger and Fontaine, *Ber.*, **60**, 1377 (1929).

(5) Benesi and Hildebrand, THIS JOURNAL, **71**, 2703 (1949).