iodine complex obtained from measurements in the visible and ultraviolet region correspond closely. This constant based on the expression of the ether in mole fraction rather than molar concentration units is 4.3, a value which is somewhat less than that (4.9) reported[2] for the diethyl ether-iodine complex in *n*-heptane at 21.5°.

DAVIS, CALIFORNIA

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

The Carbon Isotope Effect in the Acid Hydrolysis of Urea

BY JOHN A. SCHMITT AND FARRINGTON DANIELS

RECEIVED MARCH 11, 1953

Urea containing approximately 1% each of C^{13} and C^{14} was subjected to acid hydrolysis at 100° in phosphate buffer. The relative rates of decomposition were determined by mass spectrometric analysis of the carbon dioxide evolved at different time intervals during the reaction. The C^{12}-urea is hydrolyzed 10.1% faster than C^{14}-urea and 5.5% faster than C^{13}-urea. The ratio of these values agrees with theory.

The relative rates of enzymatic hydrolysis of urea at 30° and *p*H 5.0 were reported recently.[1] Values of 1.010 ± 0.001 and 1.032 ± 0.002 were obtained for $k_{C^{12}\text{-urea}}/k_{C^{13}\text{-urea}}$ and $k_{C^{12}\text{-urea}}/k_{C^{14}\text{-urea}}$, respectively.

Theoretical considerations[2,3] indicate that in competing isotopic reactions the value of $(k_{C^{12}}/k_{C^{14}} - 1)/(k_{C^{12}}/k_{C^{13}} - 1)$ should be slightly less than 2.0, although the results described for the enzymatic hydrolysis of urea, and those of some other reactions[4,5] have given a value closer to 3.0. Bigeleisen[6] has discussed these discrepancies in a recent review of the isotope effect.

In view of the obvious complication in the reaction caused by the enzyme, it was decided to carry out the hydrolysis in the absence of an enzyme. The reaction is too slow for measurement at 30°, but it is satisfactory at higher temperatures. Urea containing approximately 1% each of C^{13} and C^{14} was hydrolyzed at 100° in the presence of a phosphate buffer which gave a *p*H of 5.0 at 30°. The urea decomposed into ammonia and carbon dioxide and the relative rates of hydrolysis of urea containing C^{12}, C^{13} and C^{14} were measured by determining the isotopic ratios with the mass spectrometer, as in the preceding work. The light isotopes reacted faster and the later fractions became enriched in the heavier isotopes.

Experimental Procedure

The decomposition was carried out in a simple reaction vessel immersed in a bath at 100.0°. CO_2-free nitrogen was bubbled through the solution at the rate of 80–100 ml. per minute using a finely fritted glass tube, and the exit gas carrying along the CO_2 evolved was passed through a water condenser and then into standardized $NaOH$-$BaCl_2$ solutions.

Sixty ml. of phosphate buffer, *p*H 5.0 at 30°, was first brought to temperature and the nitrogen bubbled through for 1 hour. Then the urea in buffer was added, using a total of 10 ml. including rinses. In each experiment, 31.0 mg. of C^{14}-urea containing 1 mC. activity was diluted with Mallinckrodt A. R. urea to give approximately 1% C^{14} and total weight of urea of 75 mg.

The C^{14}-labeled urea was purchased from the Atomic Energy Commission at Oak Ridge. Successive fractions of CO_2 produced were collected in the manner described previously.[1] The mass analysis was carried out in the laboratory of Professor R. H. Burris with a mass spectrometer given by the Thomas E. Brittingham Foundation as in the earlier work.

Results

The carbon isotope ratios of the successive fractions of CO_2 collected in the two experiments are given in Table I.

TABLE I

ISOTOPIC RATIOS OF CARBON IN THE CARBON DIOXIDE RELEASED DURING THE COURSE OF THE NON-ENZYMATIC HYDROLYSIS OF UREA AT 100°

Fraction of reaction	C^{13}/C^{12}	C^{14}/C^{12}
	Experiment I	
0.000–0.168	0.010493	0.009809
.168– .335	.010624	.011047
.335– .503	.010766	.011284
.503– .671	.010923	.011551
.671– .837	.011279	.012215
	Experiment II	
0.240–0.360	0.010667	0.011034
.360– .480	.010762	.011279
.480– .600	.010905	.011515
.600– .720	.011061	.011812

The simplest way to determine the relative rates of decomposition of the C^{12}, C^{13} and C^{14}-urea from these data is by the use of an equation derived by Downes[7] which is a modified form of the one used in this earlier work on the enzymatic hydrolysis.[1]

According to this equation

$$W/N_{X^0} = \epsilon(1 - f)^{\epsilon - 1} \qquad (1)$$

or

$$\log W = (\epsilon - 1) \log (1 - f) + \epsilon \log N_{X^0} \qquad (2)$$

where

f = fraction of total reaction completed
W = $C^{\ddagger}/C^{12}$ of the CO_2 being produced at f, where $C^{\ddagger}$ is the heavier isotope, C^{13} or C^{14}
N_{X^0} = $C^{\ddagger}/C^{12}$ of the total product
ϵ = $k^{\ddagger}/k$ where $k^{\ddagger}$ and k are the specific reaction rate constants for the hydrolysis of urea for C^{13} or C^{14} and for C^{12}, respectively.

Thus, a plot of log $(1 - f)$ *vs.* log W will give a straight line with slope equal to $\epsilon - 1$. As pointed

---

[1] J. A. Schmitt, A. L. Myerson and F. Daniels, *J. Phys. Chem.*, **56**, 919 (1952).

[2] J. Bigeleisen, *J. Chem. Phys.*, **17**, 675 (1949).

[3] J. Bigeleisen and T. L. Allen, *ibid.*, **19**, 760 (1951).

[4] W. H. Stevens, J. M. Pepper and M. Lounsburg, *ibid.*, **20**, 192 (1952).

[5] P. E. Yankwich, E. C. Stivers and R. F. Nystrom, *ibid.*, **20**, 344 (1952).

[6] J. Bigeleisen, *J. Phys. Chem.*, **56**, 823 (1952).

[7] A. M. Downes, *Australian J. Scientific Research*, **5**, 521 (1952).

TABLE II

COMPARISON OF IOSOTOPE EFFECT ON THE HYDROLYSIS OF UREA

	Enzyme catalyzed 30°	Calcd. Eyring[9]	Calcd. Daniels[8]	Non-enzymatic 100°	Calcd. Eyring[9]	Calcd. Daniels[8]
$k_{C^{12}\text{-urea}}/k_{C^{14}\text{-urea}}$	1.032	1.104	1.102	1.101	1.086	1.082
$k_{C^{12}\text{-urea}}/k_{C^{13}\text{-urea}}$	1.010	1.054	1.053	1.055	1.045	1.043
$\left(\frac{k_{C^{12}\text{-urea}}}{k_{C^{14}\text{-urea}}} - 1\right)\Big/\left(\frac{k_{C^{12}\text{-urea}}}{k_{C^{13}\text{-urea}}} - 1\right)$	3.2	1.9	1.9	1.8	1.9	1.9

out by Downes, data of the type given in Table I may be used if W is taken as the $C^{\ddagger}/C^{12}$ of the CO_2 collected over a small interval and f is taken as the mean of that interval. This introduces only a small error in the determination of ϵ (within 0.5% up to 90% reaction even for fractions as large as 0.2).

Figure 1 gives a plot of log W *vs.* log $(1 - f)$ for expt. I and expt. II. The best straight lines are drawn through the points and $\epsilon - 1$ values for the slopes were calculated by the method of least squares. The averages for experiments I and II are: $k_{C^{12}\text{-urea}}/k_{C^{14}\text{-urea}} = 1.101 \pm 0.005$ and $k_{C^{12}\text{-urea}}/k_{C^{13}\text{-urea}} = 1.055 \pm 0.003$.

Conclusions

The purpose of this investigation was to find whether the ratios of competing isotopic rates of hydrolysis are the same in the non-enzymatic hydrolysis of urea as in the urease-catalyzed reaction. It was desired to find whether the presence of the enzyme is responsible for the failure of the urease-catalyzed reaction to agree with theoretical calculations, and, if so, to try to throw some light on the catalytic mechanism of the enzyme.

The results are summarized in Table II which includes the corresponding calculated values.[8–10] They show that in the absence of the enzyme the isotopic effects are much larger and the ratio of the C^{12}/C^{14} effect to the C^{12}/C^{13} effect is smaller, in much closer agreement with theory. The urease-catalyzed hydrolysis of urea was much faster, taking place with convenient velocity at 30°, whereas the non-enzymatic reaction was heated to 100° in order to obtain a similar rate. But the isotope effect in the urease-catalyzed hydrolysis is only about one-third as great in the C^{12}–C^{14} competition and less than one-fifth as much in the C^{12}–C^{13} competition.

The calculated values shown in Table II are closer to those obtained in the non-enzymatic reaction. Actually, however, too little is known about the effects of isotope substitution on bond vibration frequencies and force constants to make accurate calculations of the intermolecular isotope effect.[8] Moreover, the mechanism of reaction and the nature of the activated complex must be more thoroughly understood in order to make any calculations using Bigeleisen's approach. This is especially true of the enzyme-catalyzed reaction since very little is known about the exact nature of the enzyme–substrate complex or of the bonds broken in the rate-determining step. Enzyme kinetics are complicated, too, by the gradual change from an initial zero order to a final first-order mechanism. The activation energy is, of course, much lower for the urease hydrolysis.

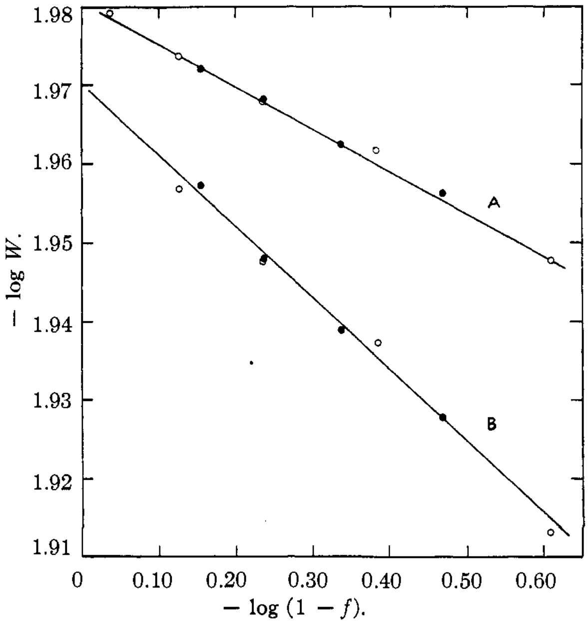

Fig. 1.—Fractionation of carbon isotopes during acid hydrolysis of urea: A, C^{12}- urea *versus* C^{13}-urea; B, C^{12}-urea *versus* C_{14}-urea: ○, expt. I, and ●, expt. II.

The reaction in phosphate buffer without enzyme gives isotope effects which are reasonably close to the values calculated using Eyring's equation. The first step in this reaction is known to be a breakdown into cyanate and ammonium ions and probably involves the breaking of a C–N bond as the rate-determining step; thus, the simplified calculations are more applicable to this reaction than to the enzymatic hydrolysis, so that it is not surprising that they check more closely.

The value of 1.8 for the ratio of $(k_{C^{12}}/k_{C^{14}} - 1)/(k_{C^{12}}/k_{C^{13}} - 1)$ is close to the predicted value of 1.9. This quantity was the object of considerable discussion at the Notre Dame Symposium on isotope effects in kinetics in June, 1952. The value of 3.2 obtained in the urease-catalyzed reaction is however, in agreement with the isotope effects of some other reactions. From the data available, it appears possible that there may be reactions in which this quantity is somewhat higher than would be predicted from the difference in mass only. Further research is necessary before these differences can be understood fully.

It is hoped that the isotope effect will be a useful

---

[8] F. Daniels, "Chemical Kinetics," Cornell University Press, Ithaca, N. Y., 1938, pp. 248–250.

[9] H. Eyring and F. W. Cagle, *J. Phys. Chem.*, **56**, 889 (1952).

[10] In these calculations the frequency of the C^{12}–N^{14} bonds in urea is taken as 1008 cm.$^{-1}$ (L. Kellner, *Proc. Roy. Soc.* (*London*), **A277**, 456 (1941)). Then the calculated frequencies for C^{13}–N^{14} and C^{14}–N^{14} are 986 and 967 cm.$^{-1}$, respectively.

tool in determining the type of chemical bond being ruptured to bring about a specified reaction.

Acknowledgment.—This work was supported in part by a grant from the Research Committee of the Graduate School with funds given by the Wisconsin Alumni Research Foundation, and in part by a Carbide and Carbon Corporation Fellowship.

MADISON, WISCONSIN

[CONTRIBUTION FROM THE EXPERIMENT STATION, HERCULES POWDER COMPANY]

Acid–Base Equilibria in Glacial Acetic Acid[1]

BY THOR L. SMITH AND JOHN H. ELLIOTT

RECEIVED JUNE 26, 1952

Values of H_0 for dilute solutions (5×10^{-4} to 5×10^{-3} *M*) of eleven strong acids in acetic acid containing 0.12% water have been measured, using indicators α-naphtholbenzein and *o*-nitroaniline. The thermodynamic constant, pK_a, for α-naphtholbenzein was found to be 0.53. At equal molarities, strengths of the acids increase in the following order: hydrochloric, methanesulfonic, sulfuric, carboxymethanesulfonic, chloromethanesulfonic, chlorocarboxymethanesulfonic, hydrobromic, perchloric, methanedisulfonic, chloromethanedisulfonic and methanetrisulfonic. H_0 values for anhydrous solutions of four monobasic acids at 5×10^{-3} *M* were measured also. From the change in H_0 caused by the addition of water, equilibrium constants for the reaction of the acid with water were calculated. Dissociation constants, K_e, of four acids in acetic acid were calculated from the conductance data of Kolthoff and Willman. Dissociation constants for perchloric and hydrochloric acids were found to be approximately 9×10^{-7} and 5×10^{-10}, respectively. Values of ΔpK_e for monobasic acids in anhydrous solutions were calculated from H_0 data and found to be in reasonable agreement with values obtained from the conductance data. The conductance and indicator data demonstrate that equilibria in acetic acid involve, primarily, undissociated species.

Introduction

The acid strengths of several strong acids and a large number of bases in glacial acetic acid have been studied by previous investigators.[2] This solvent has been used for a variety of organic reactions and for a number of analytical methods.[3] Thus, further information on strengths of strong acids, the basic strength of water, and extent of dissociation in this solvent is of considerable practical value.

The strength of an acid may be expressed only by the extent of reaction with some reference base. In water and other basic solvents, the solvent itself can be used as the reference substance. In aprotic or inert solvents like benzene, a reference base must be added so that strengths of various acids may be compared.[4] Thus, no single reference base is employed under these various conditions. Even if a single reference base is used in several solvents, its free energy will vary with the solvent, so that the measurements are not directly comparable.

A convenient and practical method of expressing the tendency of various acidic solutions to transfer a proton to a common reference substance is in terms of the acidity function, H_0, introduced by Hammett and Deyrup.[5,6] H_0 may be obtained by using the equation

$$H_0 = -\log (\mathrm{BH}^+)/(\mathrm{B}) + pK_a \qquad (1)$$

where (BH^+) and (B) are the concentrations of the acidic and the basic form of an indicator, and K_a is the thermodynamic dissociation constant for the conjugate acid of the indicator; the infinitely dilute aqueous solution is taken as the reference state.

In the present investigation, H_0 for solutions of eleven acids in acetic acid containing 0.12% water has been measured, in most cases at several concentrations (5×10^{-4}, 1×10^{-3}, 5×10^{-3} *M*) using α-naphtholbenzein and *o*-nitroaniline as indicators. Some measurements were repeated under anhydrous conditions, and from the increased acidity found, equilibrium constants for the reaction of acids with water were calculated. The dissociation constants of several common strong acids were calculated from the conductance data of Kolthoff and Willman[7] and were found to be 10^{-6} or less. From the acidity and conductance data, it is concluded that most equilibria in acetic acid involve undissociated species.

Experimental

Acids.—Reagent grade perchloric, hydrobromic, sulfuric and hydrochloric acids were used without purification.

Carboxymethanesulfonic Acid.—Barium carboxymethanesulfonate was prepared from chloroacetic acid and sodium sulfite by the method of Stillich.[8] An aqueous suspension of the barium salt was reacted with slightly less than the equivalent amount of sulfuric acid and the barium sulfate precipitate removed by filtration. The filtrate was concentrated under reduced pressure (water aspirator) at a bath temperature of 60–70°. Some crystals of barium carboxymethanesulfonate appeared and were removed by filtration. The filtrate was free of chloride and sulfate ions

---

[1] Presented before the Division of Physical and Inorganic Chemistry, 122d Meeting of the American Chemical Society, Atlantic City, New Jersey, September, 1952.

[2] (a) N. F. Hall, *Chem. Revs.*, **8**, 191 (1931); (b) A. W. Hutchison and G. C. Chandlee, THIS JOURNAL, **53**, 2881 (1931); (c) V. K. La Mer and W. C. Eichelberger, *ibid.*, **54**, 2763 (1932); (d) I. M. Kolthoff and A. Willman, *ibid.*, **56**, 1014 (1934); (e) M. A. Paul and L. P. Hammett, *ibid.*, **58**, 2182 (1936); (f) N. F. Hall and W. F. Spengeman, *ibid.*, **62**, 2487 (1940); (g) N. F. Hall and F. Meyer, *ibid.*, **62**, 2493 (1940); (h) D. S. Noyce and P. Castelfranco, *ibid.*, **73**, 4482 (1951); (i) H. Lemaire and H. J. Lucas, *ibid.*, **73**, 5198 (1951).

[3] (a) G. F. Nadeau and L. E. Branchen, *ibid.*, **57**, 1363 (1935); (b) P. C. Markunas and J. A. Riddick, *Anal. Chem.*, **23**, 337 (1951); (c) W. Seaman and Eugene Allen, *ibid.*, **23**, 592 (1951); (d) T. Higuchi and J. Concha, *Science*, **113**, 210 (1951).

[4] M. M. Davis and P. J. Schuhman, *J. Research Natl. Bur. Standards*, **39**, 221 (1947).

[5] L. P. Hammett and A. J. Deyrup, THIS JOURNAL, **54**, 2721, 4239 (1932).

[6] L. P. Hammett, "Physical Organic Chemistry," McGraw–Hill Book Co., Inc., New York, N. Y., 1940, Chapter IX.

[7] I. M. Kolthoff and A. Willman, THIS JOURNAL, **56**, 1007 (1934).

[8] O. Stillich, *J. prakt. Chem.*, **73**, 538 (1906).