the dried precipitates were stable up to temperatures of at least 300°.

Weight relations under "Experimental" and the elemental analyses gave convincing evidence for the formation of two coördination type compounds in the interaction of palladium(II) chloride and 1,2,3-benzotriazole. On the basis of the palladium content, the observed formulas are $Pd(C_6H_4NHN_2)_2Cl_2$ and $Pd(C_6H_4NHN_2)Cl_2$, and their formula weights are 415.86 and 296.74, respectively.

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On the Mechanism of Urease Action

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There are three proposed mechanisms for the hydrolysis of urea by urease in slightly acid solutions.[1] First we have the *carbonic acid mechanism* in which urea is assumed to be hydrolyzed first to carbamic acid, then to carbonic acid, and finally to carbon dioxide and water. Secondly we have the *carbamic acid mechanism* in which urea is first hydrolyzed to carbamic acid, and then the latter decomposes directly to carbon dioxide and ammonia without going through the carbonic acid stage. The weight of indirect experimental evidences in the literature seems to favor this latter mechanism. However, Sumner and Somers[1] recently proposed a third mechanism in which urea is directly hydrolyzed to carbon dioxide and ammonia without even going through the carbamic acid stage. These authors believe that the formation of ammonium carbamate in the absence of buffers is due to the recombination of the primary products CO_2 and NH_3. But it is by no means apparent just how urea can be hydrolyzed to CO_2 and NH_3 without going through the carbamic acid or carbamate stage. One possible mode of action is the reaction between a water molecule and a urea molecule under the influence of urease such that the two amide groups are simultaneously broken off and replaced by a single oxygen atom from the water molecule. But such a mechanism would require the simultaneous rupture of four bonds (two N ····· C bonds of urea and two H–O bonds of water) with the concurrent formation of three new ones (two H–N bonds of two ammonia molecules and one C=O bond of the carbon dioxide molecule), and is hence extremely unlikely. Another possible mechanism for the direct production of CO_2 is the replacement of the oxygen in urea by an unknown basic group on the urease molecule, the two amide groups are then hydrolyzed off one after the other, and finally the CO_2 is detached from the enzyme. In this way urea would indeed be converted to carbon dioxide and ammonia without going through the carbamate stage. Although there is no direct evidence to support this last mechanism, it would be risky to exclude it from our consideration in view of Sumner and Somers' suggestion. In the following discussion, this last mechanism will be referred to as the *carbon dioxide mechanism*.

It is well-known that at room temperature there is no detectable O^{18}-exchange between urea and water,[2] and that the O^{18}-exchange between carbon dioxide and water is also slow in the absence of carbonic anhydrase.[3] Consequently if ordinary urea is rapidly hydrolyzed by urease in slightly acid, H_2O^{18}-enriched water solution and the carbon dioxide liberated is immediately separated from the liquid mixture for mass-spectrometric analysis, the result should enable us to decide which one of the three mechanisms described above is correct.

For convenience of the subsequent discussion, let us introduce the new term "enrichment ratio" defined below.

$$\text{Enrichment ratio} = \frac{\text{atom \% excess of } O^{18} \text{ in } CO_2 \text{ just liberated from the urea soln.}}{\text{atom \% excess of } O^{18} \text{ in } CO_2 \text{ equilibrated with the urea soln.}}$$

The quantity in the denominator of the above ratio is determined by keeping the liberated CO_2 in contact with the liquid mixture for more than 5 hr., then separating it from the liquid and analyzing for O^{18}-content. A moment's consideration should convince us that the three mechanisms of urease action described above predict different enrichment ratios. The *carbonic acid mechanism* predicts an enrichment ratio of $^2/_3$, the *carbamic acid mechanism* predicts a ratio of $^1/_2$, and the *carbon dioxide mechanism* predicts a ratio of unity.

Water containing about 1.5 atom % of O^{18} was used as tracer in this work. The results are summarized in Table I. Three kinds of measurements were carried out. First dry sodium bicarbonate powder was mixed with excess of H_2O^{18}-enriched soln. of urea in citrate buffer (pH 5.5) in an evacuated vessel for $^1/_2$ minute at 0°. The mixture was then chilled in a Dry Ice–acetone bath, and the liberated CO_2 was sampled out for mass spectrometric analysis. The results of two such measurements listed in Table I (expts. 8 and 9) give an average O^{18} atom % of 0.22. Since the natural abundance of O^{18} is about 0.20 atom % and the water in the buffer soln. contained about 1.5 atom % in O^{18}, it is clear that under these experimental conditions the rate of O^{18}-exchange between liberated CO_2 and water is relatively slow and may be considered as negligible in all the other $^1/_2$-minute experiments at 0° described below.

In the second group of measurements the H_2O^{18}-enriched soln. of urea in citrate buffer was mixed with equal volume of H_2O^{18}-enriched soln. of urease in the same buffer for $^1/_2$ min. at 0°. The liberated CO_2 was separated and analyzed as before. The results of three determinations listed in Table I (expts. 1, 2 and 3) give an average O^{18} atom % of 0.863. In the last group of measurements, the same urea and urease soln. were mixed as before, but the liberated CO_2 was left in contact with the H_2O^{18}-enriched soln. for several hr. to reach exchange equilibrium before mass spectrometric analysis. The average O^{18} atom % in the equilibrated CO_2 obtained from four determinations (expts. 4, 5, 6 and 7 in Table I) is 1.47. Thus the

(1) For references to the literature see J. B. Sumner and G. F. Somers, "Chemistry and Methods of Enzymes," Academic Press, Inc., New York, N. Y., 1953, pp. 157–158.

(2) M. Cohn and H. C. Urey, THIS JOURNAL, **60**, 679 (1938).

(3) H. C. Urey and L. J. Greiff, *ibid.*, **57**, 321 (1935).

TABLE I

TRACER STUDIES ON THE CATALYTIC HYDROLYSIS OF UREA BY UREASE

Expt.	Reaction mixture (in H_2O^{18}-enriched 0.5 *M* citrate buffer at *p*H 5.5)	Temp., °C.	Reaction time	Atom % of O^{18} in the liberated CO_2
1	Urease + urea + buffer[a]	0	0.5 min.	0.842
2	Urease + urea + buffer[a]	0	0.5 min.	0.873
3	Urease + urea + buffer + octanol[b]	0	0.5 min.	0.873
4	Urease + urea + buffer[a]	~20	13.5 hr.	1.40
5	Urease + urea + buffer[a]	~20	8 hr.	1.52
6	Urease + urea + buffer[a]	0, 100, 20[c]	12.5 hr.	1.47
7	Urease + urea + buffer + octanol[b]	0, 100, 20[c]	6 hr.	1.50
8	Urea + buffer + $NaHCO_3$[d]	0	0.5 min.	0.224
9	Urea + buffer + $NaHCO_3$[d]	0	0.5 min.	0.219

[a] The concns. are: urea, 0.25 *M*; jack bean urease, 0.08 g./ml. [b] A droplet of octanol was added to the mixture to prevent foaming caused by the liberation of CO_2. [c] In these experiments the reactants were mixed for 1/2 min. at 0°, heated at 100° for 4.5 min. and then left at room temperature (~20°) for several hr. to reach equilibrium. [d] 25 mg. of dry $NaHCO_3$ powder was used to react with 0.6 ml. of 0.25 *M* urea in H_2O^{18}-enriched citrate buffer soln.

enrichment ratio is equal to $(0.86 - 0.22)/(1.47 - 0.22) = 0.51$, *i.e.*, approximately equal to 1/2.

Therefore it may be concluded that of the three mechanisms of urease action discussed in the literature, only the *carbamic acid mechanism* is consistent with the experimental results for slightly acid solutions obtained in the present work.

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Acid–Base Reactions in Non-dissociating Solvents *n*-Butylamine and Acetic Acid in Carbon Tetrachloride and Chloroform

BY E. ANNE YERGER AND GORDON M. BARROW

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Ion-pair structures for model systems containing a tertiary[1] and a secondary[2] amine and acetic acid have been reported recently from this Laboratory. The observation of the *n*-butylamine–acetic acid system completes this series.

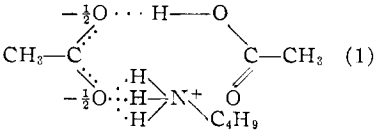
Experimental

The instrumentation,[2] and the purification of the solvents and acetic acid,[3] and the preparation of the solutions[2] have been described previously. Eastman Kodak *n*-butylamine was distilled after drying over KOH and the constant boiling center cut used. Spectra were obtained in appropriate cells for acetic acid concentrations of 0.3, 0.1, 0.02 and 0.0008 *M* with the addition of various quantities of *n*-butylamine.

The basis for the interpretation of the spectra is the same as in the diethylamine–acetic acid system previously discussed.[2] With the exception of the 5.1 μ amine salt band which will be discussed later, the behavior of the bands in the *n*-butylamine–acetic acid system parallels that of the bands in the diethylamine–acetic acid system. Thus analogous species are postulated for the present system.

In concentrated CCl_4 and $CHCl_3$ solutions an intermediate, $(AcOH)_2H_2NC_4H_9$, characterized by both 5.85 and 6.5 μ bands, is formed initially

$$(AcOH)_2 + C_4H_9NH_2 \rightleftharpoons$$

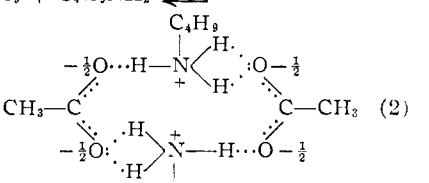


(1)

I

Structure I is suggested by analogy with the diethylamine–acetic acid system. Further addition of *n*-butylamine leads to elimination of the 5.85 μ band and replacement of the 6.5 μ band by one at 6.4 μ in both CCl_4 and $CHCl_3$. The formation of the salt dimer, $(AcOH_3NC_4H_9)_2$, with some type of hydrogen bonded bridge, depicted as structure II, is thus postulated

$$(AcOH)_2H_2NC_4H_9 + C_4H_9NH_2 \rightleftharpoons$$



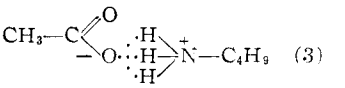
(2)

II

For the *n*-butylamine system the broad 5.1 μ band, probably an H_3N^+ deformation vibration, appears characteristic only of the intermediate, I, and is replaced by a similar band at 4.6 μ in the final product, II. This behavior contrasts the diethylamine case where the 5.1 μ amine salt band was found for both the intermediate and the final product. This shift may be interpreted as evidence for a different arrangement of the hydrogen bonding of the *n*-butylammonium ion in species II as compared with that of species I.

In the most dilute solutions the spectra and the equilibrium constants indicate direct formation of a salt monomer, $AcOH_3NC_4H_9$. In CCl_4 a carbonyl band at 5.9 μ, but no free N–H stretching band, is observed for the salt monomer, hence the formation of III is suggested

$$AcOH + C_4H_9NH_2 \rightleftharpoons$$

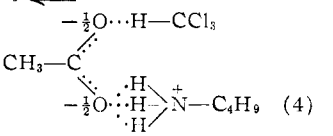


(3)

III

In $CHCl_3$ solution no carbonyl band is observed for the salt, therefore structure IV, which would be expected to show only a carboxylate band,[1,2] is postulated for the salt monomer.

$$AcOH + C_4H_9NH_2{\cdot}HCCl_3 \rightleftharpoons$$



(4)

IV

(1) G. M. Barrow and E. A. Yerger, THIS JOURNAL, **76**, 5211 (1954).
(2) E. A. Yerger and G. M. Barrow, *ibid.*, **77**, 4474 (1955).
(3) G. M. Barrow and E. A. Yerger, *ibid.*, **76**, 5248 (1954).