phenylglyoxal to mandelic acid has been found to follow the equation, rate = $0.069[C_6H_5COCHO][OH^-]$ in aqueous methanol at 25°. This implies that the reaction goes by way of an intramolecular shift of a hydrogen atom.

These facts are in agreement with an ionic mechanism for the Cannizzaro reaction based upon the intermediate formation of benzyl benzoate.

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[CONTRIBUTION FROM THE DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY, TEMPLE UNIVERSITY SCHOOL OF MEDICINE]

The Mechanism of Urease Inhibition by Urea

BY CLARA L. DEASY

Howell and Sumner[1] have shown that urease activity increases with increasing urea concentrations only if the *p*H is below 6. At *p*H values less acid than 6, inhibition of urease activity occurs with higher urea concentrations, the concentration of urea required to inhibit urease activity decreasing with increasing *p*H.

The mechanism of this inhibition is the object of this study.

Experimental

Materials.—Merck reagent urea and Baker reagent sodium acetate were used in preparing the solutions.[2] The urease, which was obtained from jack bean meal, was purified by precipitations of the crude water-soluble material, first with alcohol, and then with acetone; the final solution, which precipitated characteristic crystals of urease in the cold, was diluted for the hydrolysis experiments.

Methods. Non-Enzymatic Hydrolysis.—In expts. 1–4 (Table I) a mixture of 0.5 ml. of urea solution and 0.5 ml. of 3% sodium acetate solution was heated one hour at 100° without reflux; in expts. 5 and 6 the hydrolysis was carried out with refluxing. In expts. 7–10 a mixture of 1 ml. urea solution and 1 ml. of 2% sodium acetate solution was heated five hours at 60° in stoppered tubes. Analyses for the ammonia formed were made both by direct nesslerization of the solution and by nesslerization after the solution had been acidified and allowed to stand at room temperature.

Enzymatic Hydrolysis.—A mixture of 0.5 ml. of urea solution, 0.5 ml. of buffer solution, and 0.3 ml. of diluted urease solution was heated at 30° for five minutes. The reaction was stopped either by cooling the solution or by the addition of sulfuric acid. Ammonia analyses were carried out as in the non-enzymatic hydrolysis.

TABLE I

NON-ENZYMATIC HYDROLYSIS

Expt.	Concn. of added urea soln., %	Ammonia nitrogen direct, mg.	Ammonia nitrogen after acidification, mg.
1	1	0.01	0.04
2	3	.03	.08
3	5	.02	.19
4	10	.02	.29
5	1	.05	.10
6	10	.21	.48
7	1	.02	.02
8	3	.03	.06
9	5	.04	.07
10	10	.06	.14

Discussion

Ammonium cyanate has been shown to be an intermediate in the non-enzymatic hydrolysis of urea.[3] Since the cyanate ion is hydrolyzed to ammonia in acid solution, the amount of ammonia obtained on nesslerization after acidification of an alkaline hydrolysis mixture would be expected to be, in the limit, twice that obtained on direct nesslerization. This expectation was realized approximately when urea was hydrolyzed at 60° for five hours in a stoppered tube (expts. 7–10, Table I), but the production on acidification of much larger amounts of ammonia than the expected two-fold increase was encountered when the hydrolysis was carried out for one hour at 100° without reflux (expts. 1–4, Table I). This extra ammonia production was almost negligible if the solution was refluxed at 100° (expts. 5 and 6, Table I), but became evident again if the refluxed solution was evaporated before analysis (a 2.3-fold increase in ammonia with the 10% urea solution on acidification without evaporation, but a 3.4-fold increase (from 0.10 to 0.34 mg. ammonia nitrogen) after evaporation).

The anomalous production of the extra ammonia can be accounted for by the occurrence in the alkaline solution of ammonia in a "bound" form, as the compound $NH_3 \cdot CO(NH_2)_2$.[4] The fact that the conditions under which the extra ammonia production is encountered (higher urea concentrations, increased alkalinity, and decreased amounts of water) would be expected to favor the formation of the ammonia–urea compound, is evidence in favor of this assumption.

Direct evidence that the production of the extra ammonia on acidification was due to the presence of ammonia in a "bound" form in an alkaline solution rather than to the presence of an acid-labile intermediate was derived from experiments with solutions of ammonium hydroxide containing added urea. It was found that the color produced with such solutions was deeper on nesslerization after acidification than on direct nesslerization. The effect was intensified when the

(1) S. F. Howell and J. B. Sumner, *J. Biol. Chem.*, **104**, 619 (1934).

(2) All solutions were made up on a weight–volume percentage basis.

(3) See, for example, R. C. Warner, *J. Biol. Chem.*, **142**, 705 (1942).

(4) E. Jänecke and E. Rahlfs, *Z. Elektrochem.*, **36**, 645 (1930).

urea concentration was increased, either by direct addition of urea or by evaporation of the solution.

TABLE II

ENZYMATIC HYDROLYSIS

Expt.	Buffer	Concn. of added urea soln., %	Ammonia nitrogen direct, mg.	Ammonia nitrogen after acidification, mg.
1	1 Vol. 3%	1	0.22	0.22
2	NaOAc +	3	.29	.29
3	1 Vol. *N*	5	.28	.27
4	HOAc	10	.28	.29
5	3 Vol. 3%	1	.23	.25
6	NaOAc +	3	.30	.34
7	1 Vol. *N*	5	.29	.38
8	HOAc	10	.28	.40
9		1	.17	.17
10		3	.16	.20
11	3% NaOAc	5	.13	.20
12		10	.11	.16

In the enzymatic hydrolysis, the amount of ammonia obtained on nesslerization after acidification is greater than that obtained on direct nesslerization only in the more alkaline solutions (Table II). Since neither cyanate nor carbamate can be detected in the decomposition of urea by urease in acetate buffer,[5] and since the increased ammonia production is shown only in the more concentrated urea solutions (a fact which would not be expected if cyanate or carbamate were the cause of the increase), the increase in ammonia on acidification might be postulated as due to the presence of the ammonia–urea compound in the alkaline solution.

It should be noted that inhibition of urease activity occurs only under those conditions which give rise to increased ammonia production on acidification, and these conditions are those which would be expected to favor the formation of the ammonia–urea compound, *i.e.*, higher urea concentrations and higher alkalinity. It might be postulated, then, that the ammonia–urea compound is the real inhibitor of urease activity. Howell and Sumner's[1] results are in agreement with this postulate.

(5) J. B. Sumner, D. B. Hand and R. G. Holloway, *J. Biol. Chem.*, **91**, 333 (1931).

Summary

In an alkaline solution containing higher concentrations of urea, a portion of the ammonia present can be detected by Nessler reagent only if the solution is first acidified. This effect can be postulated as due to the presence of the compound $NH_3 \cdot CO(NH_2)_2$ in the alkaline solution.

The hydrolysis of urea by urease in acetate buffer solution is not inhibited by higher urea concentrations if the *p*H is sufficiently low; under these conditions no increase in ammonia is obtained if the solution is acidified before nesslerization. If the hydrolysis is carried out in more basic solutions, inhibition of urease activity with increasing urea concentrations is observed, and increased ammonia production on acidification before nesslerization is found at the higher urea concentrations.

The results can be explained if it is assumed that the compound $NH_3 \cdot CO(NH_2)_2$ is present at higher urea concentrations and that it acts as an inhibitor of urease activity.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF DUKE UNIVERSITY]

Condensation of Certain Esters by Means of Diethylaminomagnesium Bromide[1,2]

BY CHARLES R. HAUSER AND HOWARD G. WALKER, JR.

Diethylaminomagnesium bromide may react with carboxylic esters either at the α-hydrogen to effect their condensations or at the carbonyl carbon to produce the corresponding N,N-diethylamides. These two courses of reaction with alkyl acetates may be represented as

$$2CH_3CO_2R \xrightarrow{(C_2H_5)_2NMgBr} CH_3COCH_2CO_2R + ROH$$

$$CH_3CO_2R + (C_2H_5)_2NMgBr \longrightarrow CH_3CON(C_2H_5)_2 + Mg(OR)Br$$

We have self-condensed various esters by means of the magnesium reagent the results for which are summarized in Table I. The resulting β-keto esters were obtained free from the amide, as determined by qualitative tests for nitrogen,[3] with the exception of that from ethyl *n*-butyrate. The β-keto ester from this ester was evidently contaminated with the amide, which was not removed by simple fractionation; on ketonic cleavage in the presence of acid[4] the crude product yielded mainly di-*n*-propyl ketone and a substance assumed to be the amide. Attempts to self-condense ethyl *i*-butyrate or ethyl *i*-valerate were unsuccessful; the products appeared to consist mainly of the corresponding amide, since they failed to decarboxylate appreciably and gave strong tests for nitrogen.

(1) Paper XXXVIII on "Condensations"; paper XXXVII, THIS JOURNAL, **69**, 119 (1947).

(2) This work was supported in part by a grant from the Duke University Research Council.

(3) Moreover, no diethylamine could be detected when the product from the self-condensation of ethyl propionate was boiled with concentrated sodium hydroxide solution.

(4) Hudson and Hauser, THIS JOURNAL, **63**, 3163 (1941).