Other studies on the variation of the hydrogen-ion concentration of the acid clays upon dilution gave curves apparently identical in type with those obtained with weak acids such as acetic acid.[17]

Summary

1. Solutions of calcium and sodium hydroxides were titrated with 1% solutions of four subsoil colloidal clays. The end-points found by both the conductivity method and the hydrogen electrode were fairly definite and the curves were of the type usually obtained in titrating a strong base with a weak acid.

2. The same amounts of the colloidal acids were required to neutralize equivalent quantities of the two bases.

3. Definite breaks were found in the conductivity curves, indicating the neutralization of definite acids which in 1% solutions had concentrations ranging from 0.0027 to 0.0037 *N*.

4. The reaction between acid colloidal clays and strong bases seems to be an ordinary neutralization. Recourse to the adsorption theory seems unnecessary.

COLUMBIA, MISSOURI

[CONTRIBUTION FROM THE LABORATORY OF PHYSIOLOGICAL CHEMISTRY, UNIVERSITY OF IOWA]

STUDIES ON ENZYME ACTION. THE RELATIONSHIP BETWEEN THE CHEMICAL STRUCTURE OF CERTAIN COMPOUNDS AND THEIR EFFECT UPON THE ACTIVITY OF UREASE

BY ELBERT W. ROCKWOOD AND WILLIAM J. HUSA

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Various organic compounds have been reported as influencing the activity of urease.[1] Lövgren[2] gives a rather full review of the general properties of urease and includes a bibliography containing over 200 references. From his review of the literature Lövgren concludes that all the promoters[3] are weak acids of amphoteric electrolytes, and that their accelerating influence lies in their checking of the P_H increase.

[17] Bradfield, paper to appear in *J. Phys. Chem.*, **1923.**

[1] (a) Armstrong and Horton, *Proc. Roy. Soc.*, **85B,** 109 (1912). (b) Armstrong, Benjamin and Horton, *ibid.*, **86B,** 328 (1914). (c) Marshall, *J. Biol. Chem.*, **17,** 351 (1914). (d) Van Slyke and Zacharias, *ibid.*, **19,** 181 (1914). (e) Falk, *Biochem. Z.*, **59,** 298 (1914). (f) Jacoby and Umeda, *ibid.*, **68,** 23 (1915). (g) Jacoby, *ibid.*, **74,** 105 (1916); **84,** 358 (1917); **85,** 358 (1918). (h) Bayliss, *Arch. Néerland. physiol.*, **2,** 621 (1918); through *C. A.*, **13,** 1077 (1919). (i) Rona and György, *Biochem. Z.*, **111,** 115 (1920). (j) Wester, *Pharm. Weekblad*, **59,** 173 (1922); through *C. A.*, **16,** 1253 (1922).

[2] Lövgren, *Biochem. Z.*, **119,** 215 (1921).

[3] Pease and Taylor, *J. Phys. Chem.*, **24,** 241 (1920).

It was one of the purposes of this investigation to determine whether the promoter action reported by several investigators could be entirely explained on the basis of P_H changes, or whether there was a further effect independent of this one. Provided such an effect was found; it was believed desirable to look for a possible relationship between the chemical structure of the compounds and their action on urease, and to investigate the mechanism of the action. It was hoped that such a study would throw some light on the nature of enzyme action, and at the same time would contribute to our knowledge of promoters.[3]

Materials Used

A solid urease preparation was obtained from jack-bean meal by the method of Van Slyke and Cullen.[4] Urea was purified by recrystallization from ethanol. The compounds tested were obtained from reputable manufacturers, or from reliable research chemists, to whom we are indebted for compounds unobtainable elsewhere.

Experimental Procedure

Experiments were carried out with 0.1 *M* urea, the optimum P_H of 7.5 being maintained by use of a phosphate buffer. For the controls, 5 cc. of distilled water was introduced into a 200cc. Erlenmeyer flask, 25 cc. of *M* phosphate solution (50 volumes of *M* dipotassium and 9 volumes of *M* monopotassium orthophosphate) was added, then 15 cc. of a 2% solution of urea and 5 cc. of a 0.1% solution of enzyme, making a total volume of 50 cc. In the other flasks, the compound to be tested was first placed, in amount sufficient to make 50 cc. of a 0.001 *M* solution. Water was added, and then sufficient alkali or acid to make the aqueous solution neutral to phenolphthalein, so that it could be more readily controlled by the buffer, the given volume of dil. potassium hydroxide or sulfuric acid solution replacing part of the 5 cc. of water. The other solutions, including the buffer, were then added as in the controls. Since light is known to hasten the decomposition of urease,[2] the flasks were placed in a dark cupboard for the reaction period of approximately two hours. The variation in temperature during this time was less than 1°, and during the whole course of the work the temperature ranged from 21–28°. For analysis, a 10cc. portion of the reaction mixture was pipetted into a bottle containing 35 cc. of a saturated solution of potassium carbonate and 25 cc. of distilled water. When the action of the enzyme was thus stopped there was still an ample excess of substrate, 75 to 90% remaining unchanged. The ammonia was aerated into a mixture of 25 cc. of 0.1 *N* sulfuric acid and 50 cc. of distilled water and the excess acid determined by titration with 0.1 *N* potassium hydroxide solution, using methyl red as indicator. The time of aeration was from $3^1/_2$ to 7 hours, depending on the room temperature.

[4] Van Slyke and Cullen, *J. Biol. Chem.*, **19**, 211 (1914).

The results of Lövgren[2] were used in choosing the concentrations of urea and of the phosphates. According to his results for 0.1 *M* urea and 0.5 *M* phosphates, the formation of ammonia corresponding to 2 cc. of 0.1 *N* ammonia for each 10 cc. of the mixture caused a change of 0.1 Sörensen unit. Since the amount of promoter in 10 cc. of the mixture corresponds to only 0.1 cc. of a 0.1 *M* solution, the difference between the control and the solution containing the added substance would thus be of the order of a few hundredths of a Sörensen unit. Thus, by neutralizing the compound to be tested and employing the 0.5 *M* buffer, the effect of the added substance on the hydrogen-ion concentration was eliminated.

Experimental Data

Table I contains a summary of the results obtained with various classes of compounds. Table II summarizes the data on each compound tested. The activity of the enzyme in the presence of the added substances is expressed on the basis of the control taken as 100.

TABLE I

SUMMARIZED RESULTS GIVEN BY DIFFERENT CLASSES OF COMPOUNDS

Class	Classification	No. of compounds tested	Av. of series
I	Mono-amino-monocarboxylic acids		
	(a) NH_2 in α position	10	120
	(b) NH_2 in β position	3	103
	(c) NH_2 in γ position	1	99
II	Mono-amino-dicarboxylic acids	2	133
III	Diamino-monocarboxylic acids	1	153
IV	Diamino-dicarboxylic acids	1	133
V	Heterocyclic amino acids	3	144
VI	Derivatives of α-amino acids	3	149
VII	Amines	4	98
VIII	Amides	2	99
IX	Other compounds	18	(See Table II)

TABLE II

EXPERIMENTAL DATA FROM INDIVIDUAL COMPOUNDS

Compound	No. of det'n's	Action on basis control = 100 Min.	Max.	Av.	Av. of series
I. Mono-amino-monocarboxylic acids (a) NH_2 in α position.,					
Glycine	10	117	142	127	
Tyrosine	7	110	145	127	
l-Leucine	4	111	126	118	
dl-α-Alanine	8	116	125	121	
d-α-Alanine	2	124	125	124	
Phenylalanine	4	119	125	122	
Glucosaminic acid[a]	4	118	121	120	
Histidine methyl-ester dichloride	4	150	166	159	
Hippuric acid	6	132	154	143	149
VII. Amines					
Methylamine hydrochloride	2	98	101	100	
Diethylamine hydrochloride	2	95	96	96	
Trimethylamine sulfate	2	97	97	97	
Glucosamine hydrochloride[b]	4	100	101	100	98

Chondrosaminic acid[a]	4	114	118	115	
Gulosaminic acid[a]	4	114	121	117	
α - Amino - *n*-valeric acid	4	106	112	109	120
(b) NH_2 in β position					
β-Alanine	6	100	105	102	
d - Glucosaminoheptonic acid[a]	4	105	108	107	
l - Chondrosamino - heptonic acid[a]	4	96	104	100	103
(c) NH_2 in γ position.					
γ - Amino - *n*-valeric acid	4	97	100	99	99
II. Mono - amino-dicarboxylic acids					
Aspartic acid	14	132	165	150	
Glutamic acid	6	109	130	117	133
III. Diamino - monocarboxylic acids.					
Arginine	6	137	167	153	153
IV. Diamino - dicarboxylic acids.					
Cystine	8	116	154	133	133
V. Heterocyclic amino acids					
Histidine	6	150	170	162	
Histidine dichloride	4	140	181	159	
Tryptophan	4	109	113	112	144
VI. Derivatives of α-amino acids					
Asparagine	10	137	159	144	
VIII. Amides					
Succinamide	6	96	100	99	
Benzamide	2	98	99	99	96
IX. Other compounds					
o - Aminobenzoic acid	6	100	106	103	
m - Aminobenzoic acid	4	97	98	98	
p - Aminobenzoic acid	4	94	97	95	
Acetyl - *o*-aminobenzoic acid	4	98	102	100	
Acetyl - *m* - aminobenzoic acid	4	100	102	101	
Acetyl - *p* - aminobenzoic acid	4	100	102	100	
1 - Amino - 2 - hydroxy - 3′ bromonaphthalene hydrochloride	2	9	9	9	
Acetoxime	4	69	81	75	
Betaine hydrochloride	4	97	100	99	
Mueller's $C_5H_{11}O_2NS$, from casein	4	79	89	84	
Witte's peptone (10 mg. in 50 cc. of reacting soln.)	2	135	136	135	
Benzoic acid	2	99	102	100	
Uric acid	2	93	95	94	
Creatinine	3	98	100	99	
Creatine	3	95	99	97	
Guanidine acetic acid	4	98	100	99	
Guanidine sulfate	4	100	102	101	
Ammonium chloride, (results corr. for NH_3)	4	98	100	99	

[a] For this we are indebted to Dr. P. A. Levene.

[b] For this we are indebted to Mr. E. P. Clark.

Discussion

Relationship between Promoter Effect and Changes in Hydrogen-Ion Concentration.—The effects of the various added substances reported in the tables above cannot be explained on the basis of changes in hydrogen-ion concentration, contrary to the claims of Lövgren.[2] That the promoter action studied here is independent of such effects is assured by the fact that the substances tested were employed in 0.001 *M* concentration, neutralized and used in the presence of a 0.5 *M* phosphate buffer mixture.

Relationship between Chemical Structure and Promoter Effect Are Certain Elements Essential?—In formulating a relationship between the composition or chemical structure of the compounds and their effect upon the activity of urease it may be seen at once that the action cannot be ascribed to the presence of a single element such as nitrogen, carbon, hydrogen or oxygen, nor to the mere presence of a number of elements in a compound. True, all the promoters found in our work contain ni-

trogen, carbon, hydrogen and oxygen, but many other compounds containing these same elements exerted no favorable action.

Effect of the Amino Group Alone.—By a further analysis of the data in terms of groups it is seen that all the promoters contain an amino or substituted amino group. That the $-NH_2$ group alone is not responsible for the greater action is evidenced by the results obtained with four simple amines. As shown in Tables I and II, no promoter effect was observed, the average yield of ammonia being 98% of that from the control.

Effect of the Carboxylic Group Alone.—Jacoby and Umeda[1f] reported that no effect on the activity of urease was observed on addition of neutralized glutaric acid, $COOHCH_2CH_2CH_2COOH$. Our own experiments with benzoic acid, *o*-, *m*-, and *p*-acetylaminobenzoic acids and guanidine acetic acid also show that when care is taken to exclude changes in hydrogen-ion concentration, the carboxyl group alone has no promoter effect.

Effect of Amino and Carboxyl Groups.—Every compound which acted as a promoter contained both the amino and carboxyl groups. However, a number of compounds, both aliphatic and aromatic, containing these two groups exerted no favorable effect.

Effect of Relative Position of These Groups.—The results in Table II indicate that α-amino acids are decided promoters, β-amino acids have in some cases a slight effect, in other cases no effect, while the only γ-amino acid available had no favorable influence. In the aromatic series, the isomeric aminobenzoic acids were tested. While only the *ortho* compound showed a slight favorable effect, it seemed significant that the order was *ortho* > *meta* > *para*. The results with both aliphatic and aromatic compounds (although less marked with the latter) containing an amino and a carboxyl group indicate that the promoter action is a function of the distance between these groups, the stimulating effect increasing with decreasing proximity of the two groups.

Effect of Substituted Amino and Carboxyl Groups.—The methyl ester of histidine gave the same results as histidine itself, a result which indicates that esterification of the carboxyl group in a promoter does not decrease the favorable effect. Hippuric acid was a marked promoter; this result shows that the benzoyl group may replace one hydrogen of the amino group without decreasing the promoter action.

Effect of a Second Carboxyl Group and its Position.—From the data in Tables I and II it appears that the second carboxyl group slightly increases the promoter effect. In one experiment in which the two compounds were compared directly against the same control, the result for aspartic acid was 133, and that for glutamic acid 129. Since in glutamic acid the second carboxyl is separated from the amino group by one more carbon atom than in aspartic acid, it is possible that the promoter effect may depend somewhat on the proximity of the second carboxyl group.

Effect of a Second Amino Group.—A second amino group was found to increase the promoter effect. The results with arginine (guanidine-α-aminovaleric acid) averaged 153, a value which is considerably higher than the average for α-mono-amino-monocarboxylic acids.

Effect of Second Amino and Carboxyl Groups in the Same Compound.—From the results obtained with cystine it appears that the second amino group does not increase the promoter effect if there are two carboxyl groups, while a second carboxyl group does not increase the promoter effect if there are two amino groups in the compound. There is a possibility, however, that the two thio groups of cystine may influence the promoter effect.

Effect of a Heterocycle Containing Nitrogen.—Tables I and II indicate that α-amino acids having in addition a nitrogenous heterocycle give, on the average, a somewhat greater promoter effect than simple α-amino acids.

Effect of Length of Chain.—There is some indication that in aliphatic α-amino acids the promoter effect decreases with lengthening of the carbon chain. Thus the average value obtained for $CH_2(NH_2)COOH$ was 127; for $CH_3CH(NH_2)COOH$, 121; and for $(CH_3)_2CHCH_2CH(NH_2)COOH$, 118.

Effect of Optical Isomers.—Jacoby and Umeda[1f] working with urease without, however, eliminating changes in hydrogen-ion concentration observed no difference in action between *dl*-alanine and *d*-alanine, or between *dl*-glutamic acid and *d*-glutamic acid. In our experiments, in which effects of hydrogen-ion concentration played no part, the promoter effects of *dl*-alanine and *d*-alanine were also found to be practically equal.

Compounds Having No Promoter Action

Effect of Ammonium Chloride.—Armstrong and Horton[1a] and other investigators have reported a favorable action of ammonium chloride on urease. In our work, changes in hydrogen-ion concentration being eliminated and correction made for the ammonia from the ammonium chloride, no such greater action has been found. Thus the favorable effect of ammonium chloride reported by others is not the specific effect demonstrated in this investigation.

Effect of Guanidine Derivatives.—Because of the apparent favorable influence of the guanidine group in arginine, other guanidine derivatives were tested. Guanidine sulfate, guanidine acetic acid, creatine and creatinine gave no promoter effect. To cover the field an amino-naphthol, an oxime and various other compounds were tried (see Table II), but no promoter effect was observed.

Effect on Activity of Enzyme as a Test for the α-Amino Group

From the results of our work it appears that the effect of a compound on the activity of urease might furnish valuable presumptive evidence as

to the presence of an amino group in the α-position to a carboxyl group. An opportunity to apply this method presented itself in connection with the isolation of a new amino acid from casein by Mueller.[5] He reported that the empirical formula of the compound was $C_5H_{11}O_2NS$, and that tests were obtained showing the presence of an amino group and a carboxyl group. The results obtained with this substance[6] showed a depressing effect on the activity of the enzyme, the average being 84% of the controls. It would appear that this new sulfur-containing amino acid is not an α-amino-monocarboxylic acid of the cystine type.

Importance of Protein Associated with Enzymes

Enzymes seem to be of a protein nature or to occur associated with protein. When the protein is removed from an enzyme solution by coagulation with heat or other means the activity is lost. For this reason it is often stated that enzymes are proteins, or something more complex containing protein.

Van Slyke and Cullen[4] found that their soya urease preparation contained a few per cent. of ash, the organic matter being about $^2/_3$ protein and $^1/_3$ carbohydrate. Since three days' digestion with trypsin or papain did not markedly injure the activity of the enzyme it seemed to them unlikely that the urease was of a protein nature.

Jacoby and Umeda,[1f] working with soya urease without controlling the hydrogen-ion concentration found the activity of the enzyme to be increased by glycyltryptophan, Witte's peptone and casein. Our experiments indicated that Witte's peptone exerts a promoter action comparable to that shown by α-amino acids.

Jacoby and Sugga[7] reported that soya urease was not appreciably injured by dialysis, or by treatment with trypsin or papayotin. When the urease preparation was first treated with papayotin and then dialyzed, they observed a marked diminution in the activity of the urease.

Their results may be explained as follows. It has been shown that α-amino acids, peptones and proteins serve as promoters of the catalytic action of the urease. It would, therefore, be reasonable to expect a fall in activity of the enzyme preparation when the compounds containing α-amino groups are removed. Proteins being, as a rule, non-diffusible, dialysis alone might not appreciably affect the action of the urease. Further, since our work has shown that α-amino acids and peptones are marked promoters, it is evident that the breaking down of the proteins into peptones and amino acids by treatment with trypsin or papayotin should likewise not change the rate of fermentation. But if the urease preparation is dialyzed after treatment with papayotin, the protein having been changed

[5] Mueller, *J. Biol. Chem.*, **55**, xv (1923).

[6] Kindly furnished by Dr. Mueller.

[7] Jacoby and Sugga, *Biochem. Z.*, **69**, 116 (1915).

into diffusible amino acids which would be removed by the dialysis, a diminution in activity of the urease should be expected. The results of Jacoby and Sugga[7] may thus be explained on the basis that the protein part of an enzyme preparation, by its promoter action, accounts for part of the activity of the enzyme.

The Mechanism of the Promoter Effect

Various explanations have been given[8] of the manner in which the activity of enzymes is increased by added substances. Sherman and Naylor[9] conclude that the influence of amino acids on amylases "may be attributed either to a direct 'activating' effect dependent upon the structural nature of these substances as α-amino acids, or to conservation of the enzyme by retarding its hydrolysis."

In dealing with urease one must also consider whether an added substance such as an amino acid might not interact with the enzyme to form ammonia. Likewise, the ureolytic power of bacteria from the air must be remembered, although solutions of purified urea do not offer a medium favorable to microörganisms which can readily hydrolyze the urea in urine. To learn whether there were any secondary effects, the following experiment was carried out. The concentrations in the reacting mixture were 0.1 *M* urea, 0.01% urease preparation and 0.05% *dl*-α-alanine. No buffer was used and the ammonia could thus be determined by direct titration with alkali after the reaction had been stopped by the addition of standard sulfuric acid solution. To allow maximum opportunity for possible bacterial action and other effects the solutions described below were allowed to act for two days instead of for the usual time of two hours.

TABLE III

Substances present besides distilled water	Cc. of 0.1069 *N* NH_3 generated in 10cc. sample	Substances present besides distilled water	Cc. of 0.1069 *N* NH_3 generated in 10cc. sample
Enzyme	0.0	Urea + α-alanine (neutr.)	0.1
Urea	0.0	Urea + enzyme	3.1
α-Alanine (neutralized)	0.0	Urea + enzyme + α-alanine (neutr.)	15.2
Enzyme + α-alanine (neutr.)	0.0		

These results indicate that no ammonia is formed by hydrolytic or bacterial decomposition of urea, urease or α-alanine alone, or by interaction of the enzyme with the amino acid. Urea in the presence of α-alanine undergoes a small but measurable change, which may be due to a slight catalytic activity of the amino acid, or to bacterial action due to improvement of the medium for growth of microörganisms. In any case the effect could

[8] Ford, *J. Soc. Chem. Ind.*, **23**, 414 (1904). Donath, *Hofmeister's Beitr.*, **10**, 390 (1907). Ref. 1i. Sherman and Walker, THIS JOURNAL, **43**, 2461 (1921). Sherman and Caldwell, *ibid.*, **43**, 2469 (1921); **44**, 2923, 2926 (1922). Biedermann, *Arch. Néerland. Physiol.*, **7**, 151 (1922); through *C. A.*, **17**, 564 (1923).

[9] Sherman and Naylor, THIS JOURNAL, **44**, 2957 (1922).

scarcely be observed in a two-hour reaction period and it is by no means an important part of the promoter effect reported in this paper. A similar experiment with histidine, in the presence of the phosphate buffer, confirmed the conclusion that there is only a negligible amount of ammonia formed by the action of the amino acid alone on urea. The α-alanine does not catalyze the hydrolysis of urea to an important extent but does increase the catalytic activity of the urease, and we are thus dealing with an undoubted promoter action.[3]

It is well known that aqueous solutions of enzymes lose their activity on standing at room temperature and that at higher temperatures this decomposition proceeds more rapidly. Our experiments showed that glycine in 0.001 *M* concentration protected urease from heat destruction at 75° to a marked degree. Experiments were then carried out to determine whether this protective action was great enough at room temperature to account for the promoter action reported in this paper. In one set of experiments the decomposition on standing in the absence of substrate of an aqueous solution of the enzyme alone and with an amino acid present was studied. In the other set of experiments the rate of decomposition of the enzyme with and without amino acid was determined in the presence of the substrate, that is, while the enzyme was functioning as a catalyst.

Decomposition in Absence of Substrate.—An experiment was conducted using the same procedure and the same concentrations as in the tests of the effect of added substances. As a control the activity of a freshly prepared enzyme solution was determined by a two-hour digestion after standing for 0 hours and 15 hours, respectively. The activity was also determined with amino acid present during digestion only, as well as with amino acid present during both periods. The results in cc. of 0.0653 *N* ammonia were as follows.

TABLE IV

Time of standing Hours	Control	*dl*-α-Alanine added just before digestion	*dl*-α-Alanine present both before and during digestion
0	4.73	5.45	5.39
15	3.25	3.67	3.87
% Decomp. in 15 hours	31	33	30

The decomposition of urease in presence of the amino acid was from 1 to 3% less than in the control over a 15-hour period, while the promoter action of the amino acid for a two-hour digestion was 14% with an enzyme solution tested immediately after dissolving the urease and 19% with an enzyme solution which had been standing 15 hours. Thus, while a slight protective action of the amino acid has been demonstrated, most of the increase in catalytic activity of the enzyme must be attributed to a direct promoter action.

Decomposition in Presence of Substrate.—When urease is added to a

solution of urea, and the amount of ammonia formed is determined for successive one-hour intervals, it is found that less urea is hydrolyzed during the second hour than during the first hour. This apparent decrease in rate of change may be ascribed to three causes: (1) to the decrease in concentration of substrate, (2) to a retarding effect of the products formed, and (3) to decomposition of the enzyme. Instead of expressing the experimental results in terms of the amount of ammonia formed in a given time, the length of time necessary to form a given quantity of ammonia may be obtained by interpolation on a curve in which the amount of ammonia formed is plotted against time. By this procedure the decrease in concentration of the substrate and any effects of the products formed will be the same in the control and in the parallel determinations, and the extent of decomposition of the enzyme may be closely estimated by comparing the percentage increase in time required in the two cases to bring about equal increments of change in successive intervals. That is, if in one digestion the enzyme is decomposing faster than in another, more time, proportionately, will be required for bringing about the second increment of change in the first case than in the second.

Experiments were carried out with *dl*-α-alanine and with histidine, using the same procedure and same concentrations as were used in the tests of the effect of various added substances.

TABLE V

EXPT. 125

	Cc. of 0.0653 *N* NH_3 formed in			Time required to form 1st 3cc. of 0.0653 *N* NH_3	Time required to form 2d 3cc. of 0.0653 *N* NH_3
	1 hr.	2 hrs.	3 hrs.	Min.	Min.
Control.....	2.63	4.84	6.72	70	88
dl-α-Alanine added..	2.83	5.12	7.18	64	81

In the control the second period required 18 minutes longer than the first, indicating on the average 26% less enzyme activity during the second period. In the presence of *dl*-α-alanine 17 minutes longer was required, showing a 27% decrease in rate of enzyme action during the second period. These results show that the enzyme was being decomposed as rapidly in the presence of amino acid as when no amino acid has been added. Comparing the two first periods a decrease in time from 70 to 64 minutes or 9% was obtained by the addition of *dl*-α-alanine. Since

TABLE VI

EXPT. 126

	Cc. of 0.0653 *N* NH_3 formed in			Time required to form 1st 2.5 cc. of 0.0653 *N* NH_3	Time required to form 2d 2.5 cc. of 0.0653 *N* NH
	1 hr.	2 hrs.	3 hrs.	Min.	Min.
Control.....	2.25	4.13	5.85	69	82
dl-α-Alanine added..	2.40	4.49	6.31	62	75

the destruction of enzyme in each case was practically the same, the promoter action cannot be explained on the basis of a prevention of destruction of the enzyme.

Thirteen minutes longer was required for the second period in each case, indicating on the average a 19% slower rate of action in the control and a 21% slower rate of action in the presence of *dl*-α-alanine during the second period. These results indicate that the enzyme is destroyed as rapidly while exerting its catalytic effect in the presence of *dl*-α-alanine as in its absence. The amino acid brought about a 10% decrease in time required for the first period (as is seen by comparing the two first periods), and this promoter action, is, therefore, due to an increased activity of the enzyme brought about by the amino acid.

The results obtained with histidine led to the same conclusion as those with *dl*-α-alanine. The experiments thus disprove the hypothesis that the promoter effect is due chiefly to prevention of decomposition of the enzyme. On the other hand, the results support the view that the promoters directly facilitate the interaction of the enzyme with the substrate.

Summary

1. It has been found that certain compounds exert a promoter effect on the catalytic activity of the urease of the jack bean. It was also shown that some compounds have an inhibitory action. These effects are independent of changes in hydrogen-ion concentration and do not result from the attainment of a concentration of electrolytes more favorable or unfavorable to the action of the enzyme.

2. The promoter effect is related to the presence of amino and carboxyl groups in the same compound, since each promoter contained both these groups, while compounds containing only one of them were not promoters.

3. The results with both aliphatic and aromatic compounds containing an amino and a carboxyl group indicate that the promoter action is a function of the distance between these groups, the stimulating effect increasing with decrease in their proximity. All α-amino acids tested are marked promoters, β-amino acids have a slight promoter action, and γ-amino acids have no effect. The isomeric aminobenzoic acids, in order of decreasing promoter effect are: *ortho* > *meta* > *para*.

4. In α-amino acids, replacement of one hydrogen of the amino group by benzoyl, or esterification of the carboxyl group does not diminish the promoter action.

5. A second carboxyl group in an α-amino acid appears to increase the promoter effect slightly.

6. A second amino-group in an α-amino acid was found to increase the promoter action.

7. A second amino group in a compound already containing one amino and two carboxyl groups, or a second carboxyl group in a compound already containing one carboxyl and two amino groups does not appear to increase the promoter effect.

8. α-Amino acids with a nitrogenous heterocycle give, on the average, a somewhat greater promoter effect than simple α-amino acids.

9. There is some indication that in aliphatic α-amino acids the promoter effect decreases with lengthening carbon chain.

10. In α-amino acids, optical isomers do not differ in promoter effect.

11. Ammonium chloride has no promoter action when changes in hydrogen-ion concentration are eliminated and correction is made for the ammonia it contains. Thus the favorable effect of ammonium chloride reported by others[1a] is not the specific effect demonstrated in this investigation.

12. From the results of our work it appears that the effect of a compound on the activity of urease may furnish valuable presumptive evidence as to the presence of an amino group in the α position to carboxyl.

13. The experiments which show the promoter action of α-aminocarboxylic acids and of peptones indicate that the protein part of enzyme preparations is an important factor in the action of the enzyme because its promoter action accounts for part of the activity of the enzyme.

14. The experiments tend to disprove the hypothesis that the promoter effect is due chiefly to prevention of decomposition of the enzyme. On the other hand, the results support the view that the promoters directly facilitate the interaction of the enzyme with the substrate.

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[CONTRIBUTION FROM THE LABORATORIES OF GENERAL CHEMISTRY, UNIVERSITY OF WISCONSIN]

A NEW METHOD FOR THE DETERMINATION OF ACETIC ACID IN ACETIC ANHYDRIDE

BY JAMES H. WALTON AND LLOYD L. WITHROW

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Acetic anhydride has become so important a reagent in organic synthesis, that a rapid and accurate method for determining its chief impurity, acetic acid, is very desirable. In a recent investigation by E. R. Schierz[1] and the senior author of this paper a method for determining this substance was indicated.

When formic acid is added to acetic anhydride, it is broken up quantitatively according to the equation $HCOOH = H_2O + CO$. At ordinary temperatures the reaction proceeds so slowly that it is practically unmeasurable. It was found by Schierz that this reaction is catalyzed by

[1] Schierz, THIS JOURNAL, **45**, 455 (1923).