The product was identical with *scyllo*quercitol.[12a] Admixture of authentic *scyllo*quercitol prepared by us from *scyllo*inosose did not depress the m.p. A portion was converted to the pentaacetate, m.p. 193–194°. Identity of the two acetates was demonstrated by X-ray powder pictures.

D,L-(1,2,4)-R-Quercitol ("D,L-*vibo*Quercitol," XVI) from Bromo*vibo*quercitol-*A*.—Bromo*vibo*quercitol (m.p. 171°) was catalytically debrominated as above. Traces of nickel ion were removed from the hydrogenation filtrate by the use of Amberlite IR-120 (H^+) resin until a negative dimethylglyoxime test was obtained. The resulting acidic solution was neutralized by treatment with IR-4B resin, filtered, and the filtrate vacuum-distilled to a small volume.

On addition of 25 ml. of absolute ethanol, crystals separated. The solvent was removed by vacuum-distillation, giving 0.51 g. (77%) of colorless crystals, m.p. 154–158°. Recrystallization from 95% ethanol gave 0.32 g. (48%) of crystals, m.p. 161–163°.

The product appears to be identical with the D,L-*vibo*-quercitol of Posternak,[12b] reported m.p. 161–163°.

A portion acetylated with acetic anhydride gave D,L-*vibo*-quercitol pentaacetate of m.p. 112–113°, reported[12b] m.p. 113–114°.

Reaction of Bromo*scyllo*quercitol-*A* with Sodium Hydroxide.—A 0.4861-g. portion of bromo*scyllo*quercitol (m.p. 224° dec.) was dissolved in sufficient 0.1611 *N* sodium hydroxide to make 50.00 ml. Aliquots of 5.00 ml. were withdrawn at intervals for titration with 0.1047 *N* hydrochloric acid (phenolphthalein), with the results shown:

Time, min.	NaOH consumed, moles/mole	Time, min.	NaOH consumed, moles/mole
0	0	73	0.95
8	.49	126	0.99
28	.74	268	1.00
45	.88	1455	1.02

The last five neutralized samples were combined, and deionized by successive treatment with Amberlite IRA-400 and IR-120 (H^+) resins. The filtered solution on vacuum distillation left a colorless oil as residue. A portion of the oil was dissolved in ethanol and gave a negative test for carbonyl groups with dinitrophenylhydrazine–phosphoric acid reagent.[21]

The remainder of the oil crystallized after standing for three weeks with ethanol, m.p. 146–151°. The crystals gave a negative Beilstein halogen test, and are rather soluble in water. The exact nature of this compound has not yet been determined; presumably it is an epoxycyclohexanetetrol.[22]

(21) C. D. Johnson, THIS JOURNAL, **73**, 5888 (1951).

(22) Cyclohexene oxide is quite stable with dilute aqueous alkali (partly because of low solubility), but the polyhydroxyepoxide presumably formed here would perhaps be more reactive. It might be hydrolyzed to an inositol before isolation is possible.

TORONTO, CANADA

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

Some Carboxypeptidase–Substrate Relationships[1]

BY EDWARD RONWIN[2]

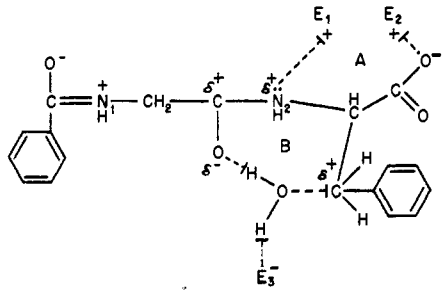
RECEIVED APRIL 11, 1953

In accordance with the dipositive-bond theory, N-*p*-toluenesulfonyl-DL-phenylalanine and N-chloroacetyl-DL-aspartic acid were found to be resistant to the enzyme. Several representatives of N-chloroacetylated, N-trichloroacetylated and N-hippurylated amino acids have been subjected to the action of the enzyme for the first time. There are indications of a substrate inhibition in the case of chloroacetyl-DL-tyrosine. At first approximation, the trichloroacetate ion is a strong inhibitor. Contrary to the notion that the greater the acid strength of the acyl moiety, the greater the rate of hydrolysis; the trichloroacetyl derivatives have been found to be much poorer substrates than the corresponding chloroacetyl derivatives of amino acids. It was unexpectedly observed that hippurylleucine is a better substrate than hippuryltyrosine. The results, in general, were as anticipated and demonstrate that hippuryl derivatives of amino acids are, thus far, the most potent synthetic carboxypeptidase substrates.

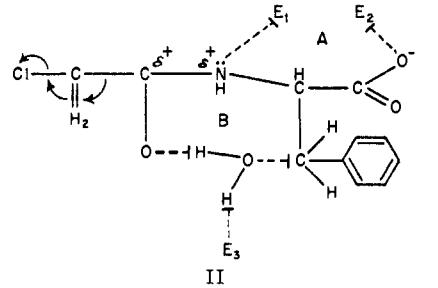
Introduction

The recently suggested dipositive-bond theory[3] serves as the stimulating principle of these studies. The basic tenets of this theory are two in number: (1) the susceptible bond is ruptured or considerably weakened as a result of the creation of a dipositive charge situation at the hydrolyzable link which is in turn caused by the nature of the bonding of the substrate to the enzyme and (2) the formation of a fruitful ES complex, with the ensuant dipositive situation, involves the simultaneous creation of two rings in the union of enzyme and substrate. This is illustrated by I for N-substituted dipeptide substrates and by II for the N-acylated amino acid substrates of the enzyme (beef pancreatic carboxypeptidase) that is being considered here.

According to the dipositive-bond theory, the contribution to substrate susceptibility of the group attached to the nitrogen atom of the antipode am-



I[4]



II

(1) Part of this work was performed during the tenure of a Life Insurance Medical Research Fellowship. The manuscript was originally received June 11, 1952.

(2) Department of Physiology, Tufts Medical College, Boston 11, Mass.

(3) The theory and its application to several enzymes is discussed in detail in the Ph.D. thesis of the author. Also, *Enzymologia*, in press.

(4) The symbol, ·····|, represents any bond between oppositely charged groups on the enzyme and substrate. It is always written with the head adjacent to the positive area.

ino acid of a carboxypeptidase substrate is qualitatively the same regardless of the nature of the groups occupying the other positions of the substrate. Therefore, in a comparison of any two or more series of substrates, these groups should exhibit parallel activity-stimulating influences. To test the validity of this notion, the reaction between the enzyme and eleven chloroacetylated, seven trichloroacetylated and five hippurylated amino acids was followed. Prior to this work the only series for which extensive data (but under considerably varying conditions) had been quoted was that of the carbobenzoxyglycyl derivatives of amino acids.[5]

In addition, *p*-nitrocarbobenzoxyglycyl-L-leucine was run to obtain an idea of the effect of the *p*-nitro substitution in the carbobenzoxyglycyl group on the hydrolytic susceptibility of the substrate.

N-*p*-Toluenesulfonyl-DL-phenylalanine was subjected to the action of the enzyme to determine whether or not a sulfonamide link is hydrolyzed.

Experimental

Compounds.—N-*p*-Toluenesulfonyl-DL-phenylalanine.—This compound was prepared by the method of McChesney and Swann.[6] Four recrystallizations from 60% ethanol were required before a 38% yield of a pure compound, m.p. 133–134° (lit. 133–134°),[6] was obtained.

Anal. Calcd. for $C_{16}H_{17}N_1O_4S_1$: neut. equiv., 319. Found: neut. equiv., 320.

The synthesis of the chloroacetylated, trichloroacetylated and hippurylated amino acids was performed by a direct method of acylation.[7]

Thanks are due to Mr. Duane Gish for his generous gift of pure *p*-nitrocarbobenzoxyglycyl-L-leucine.

Standard Conditions.—All ES reactions were performed under the following conditions: 25°, *p*H 7.5, 0.27 ionic strength, 0.04 *M* phosphate buffer made 0.1 *M* with respect to LiCl, 1 ml. reaction volume and 0.025 *M* initial substrate concentrations. These conditions were chosen to enhance the reliability of comparison with earlier work.

Enzyme.—The enzyme sample used was a three times crystallized product of the Worthington Co., Freehold, N. J. Concentrated solutions of the enzyme in 5% lithium chloride were prepared within three days of use. These solutions were found to lose no activity during this period.

Enzyme–Substrate Reaction Procedure.—Sufficient substrate to yield a 0.025 *M* solution with respect to the L-form in 1.5 ml. is dissolved in 600 λ of 0.1 *M* phosphate buffer (*p*H 7.5) containing 0.25 *M* lithium chloride. The *p*H is readjusted to 7.5 and the solution is diluted to a desired volume. Then, an aliquot of this solution is so chosen such that, upon addition of the concentrated enzyme solution, the total volume of the enzyme–substrate reaction medium will be one ml. having a substrate concentration of 0.025 *M* and a buffer concentration of 0.04 *M* plus 0.1 *M* lithium chloride. The remainder of the substrate solution is incubated as a substrate control and an enzyme control consisting of the proper ratio (on one-tenth scale) of enzyme, water and buffer is simultaneously incubated. Before addition of the enzyme to the reaction solution, the solution and controls are permitted to come to temperature equilibrium with the water-bath at 25 ± 0.01°.

With rare exceptions, a 20 λ aliquot was periodically withdrawn from the enzyme–substrate solution and was pipetted into a photometer tube containing 40 λ of 0.2 *M* citrate buffer at *p*H 5. This was followed by 140 λ of doubly distilled water. This stopped the reaction immediately. The progress of the ES reaction was then followed by subjecting the test samples to analysis using the quantitative ninhydrin method.[8]

(5) H. Neurath and G. W. Schwert, *Chem. Revs.*, **46**, 69 (1950).

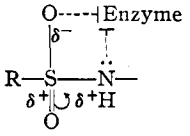
(6) E. W. McChesney and W. K. Swann, Jr., THIS JOURNAL, **59**, 1116 (1937).

(7) E. Rouwin, *J. Org. Chem.*, **18**, 127 (1953).

(8) S. Moore and W. H. Stein, *J. Biol. Chem.*, **176**, 367 (1948).

Results and Discussion

N-*p*-Toluenesulfonyl-DL-phenylalanine.—After 76.5 minutes, under the influence of an enzyme concentration of 0.014 mg. N/ml., other conditions being standard, except that the reaction mixture contained 20% methyl alcohol by volume, N-*p*-toluenesulfonyl-DL-phenylalanine proved resistant to carboxypeptidase. Though the possibility of steric hindrance is not excluded, this result is in accordance with the theory, since the creation of a dipositive situation at the susceptible bond would be nullified by a negativity contribution from the oxygen atom that is attached to the sulfur atom of the susceptible link but is not engaged in an ES complex III.



III

N-Chloroacetyl-DL-aspartic Acid.—In the postulated complex (I or II) for carboxypeptidase, position E_3 has been assigned a negative character. Hence, any compound with a side chain that possesses a unit, *exposed* negative charge would be expected to be resistant to the formation of the ES complex. This expectation is supported by the observation that N-chloroacetyl-DL-aspartic acid is completely devoid of substrate activity,[9] under conditions (0.0777 mg. N/ml., standard conditions, 120 minutes of incubation), which yield measurable activity for analogs which have side chains with one more and one less carbon atom, but lacking any *exposed* charge type.

N-Chloroacetyl-DL-tyrosine.—Putnam and Neurath[10] introduced an empirical correction factor (1) to the experimentally obtained first-order rate constant for chloroacetyltyrosine which yielded values that permitted the calculation of acceptable proteolytic coefficients

$$k' = k + k_2x \qquad (1)$$

k is the experimentally determined first-order rate constant; k_2 is the empirical correction factor equal to 3×10^{-4} and x is the fraction of substrate that is hydrolyzed. k' is the corrected specific rate constant. This formula has been applied in this work.

The substrate was run at three initial concentrations: 0.025, 0.045 and 0.05 *M*. The *uncorrected* results are presented in Fig. 1. After adjustment to equal enzyme concentrations, a gradual rate increase is observed with decreased substrate concentration.

Since it is reasonable to expect that the same relative degree of inhibition by the chloroacetate ion would exist regardless of the magnitude of the initial substrate concentration, the higher susceptibility of chloroacetyltyrosine to carboxypeptidase at 0.025 *M* initial concentration as compared to that at 0.05 *M* is attributable to the presence of substrate inhibition at the higher initial concentration. Similar substrate inhibition has been recently reported in the reaction between the enzyme

(9) At the *p*H of the reaction, both carboxyl groups of the substrate are completely dissociated.

(10) F. W. Putnam and H. Neurath, *J. Biol. Chem.*, **166**, 603 (1946).

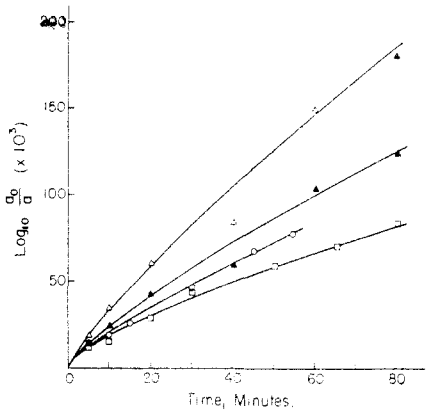


Fig. 1.—Rate of hydrolysis *versus* initial substrate concentration for N-chloroacetyl-DL-tyrosine: □, 0.05 *M*, (E) = 0.0018 mg. N/ml.; O, 0.045 *M*, (E) = 0.0018 mg. N/ml.; △, 0.025 *M*, (E) = 0.00259 mg. N/ml.; ▲, 0.025 *M*, (E) corrected to 0.0018 mg. N/ml.; other conditions standard.

and the carbobenzoxyglycyl derivatives of phenylalanine and tryptophan.[11]

Thus, in this case, the empirical correction factor (1) is a composite correction for both chloroacetate ion and substrate inhibition. Also, consideration of this effect constituted a factor in setting the initial substrate concentration in all other runs at 0.025 *M*.

Insertion of the corrected first-order constants, *k'*, into the formula for first-order rate kinetics yields a modified rate expression in terms of decimal logarithms. A plot of the resulting data for chloroacetyl-DL-tyrosine, accompanied by a plot constructed from the data for the same substrate as quoted by Putnam and Neurath,[10] is presented in Fig. 2.

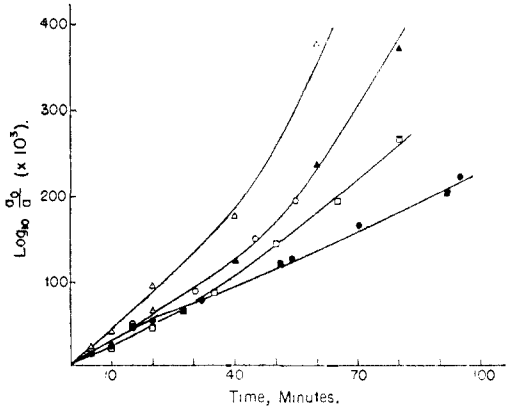


Fig. 2.—Hydrolytic rate of N-chloroacetyl-DL-tyrosine as measured by modified first-order kinetics *versus* initial substrate concentration: □, 0.05 *M*, (E) = 0.0018 mg. N/ml.; O, 0.045 *M*, (E) = 0.0018 mg. N/ml.; △, 0.025 *M*, (E) = 0.00259 mg. N/ml.; ▲, 0.025 *M*, (E) corrected to 0.0018 mg. N/ml.; other conditions standard; results of Putnam and Neurath: ●, 0.05 *M*, (E) = 0.0036 mg. N/ml. corrected to 0.0018 mg. N/ml.; ■, 0.05 *M*, (E) = 0.0018 mg. N/ml.; conditions: 40°, *p*H 7.7, 0.02 *M* phosphate buffer plus 0.05 *M* LiCl.

(11) R. Lumry, E. L. Smith and R. R. Glantz, THIS JOURNAL, **73**, 4330 (1951).

Since first-order kinetic rates are theoretically independent of initial substrate concentrations, all the curves, corrected to equal enzyme concentrations should be identical. This is the case with the runs of the present work at 0.025 and 0.045 *M* initial substrate concentrations. Only a slight deviation occurs in the case of the 0.05 *M* initial substrate concentration experiment. The values of Putnam and Neurath over the first 30 minutes of the reaction agree very well with each other and with the new data. The deviations in the latter periods of the reaction probably reflect changes due to the considerably different reaction conditions employed by these authors (for example, they operated at 40° in distinction to the 25° reaction temperature used in this work). These results attest to the validity of employing the empirical correction factor to describe the reaction kinetics of the chloroacetylated derivatives.

Other Chloroacetylated Amino Acids.—The inhibition by chloroacetate ion was present in the reactions of all the other chloroacetyl derivatives. The empirical correction equation (1) was found to yield satisfactory results for all the other representatives of this series that were tested.

N-Trichloroacetylated Amino Acids.—Trichloroacetyl-DL-tyrosine, as the other trichloroacetylated amino acids, does not follow first-order kinetics in its reaction with the enzyme. Figure 3 contains a plot of the rate–time curve for the two runs performed with this substrate. After the first five minutes of the reaction, the rate experiences a sharp decline, which indicates that an inhibition by the trichloroacetate ion (as was expected) is a factor in the hydrolysis of this compound. Application of the same empirical correction equation (1) used for the chloroacetyl derivatives yields a uniform first-order rate constant except for the value at the five-minute point of the reaction.

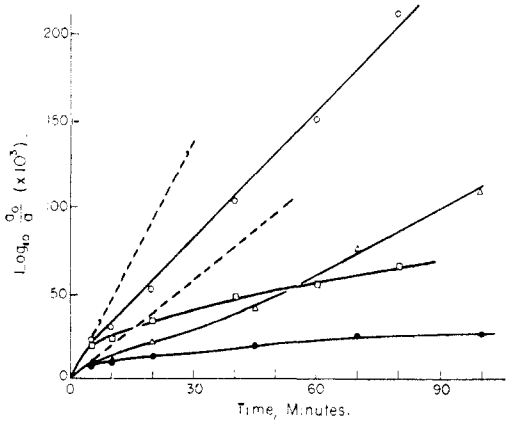


Fig. 3.—Rate of hydrolysis of N-trichloroacetyl-DL-tyrosine: ●, experimental rate curve, (E) = 0.014 mg. N/ml., other conditions standard: △, modified rate curve; □, experimental rate curve, (E) = 0.1295 mg. N/ml., other conditions standard; O, modified rate curve.

The correction equation (1) when applied to the trichloroacetyl derivatives of L-leucine and DL-isoleucine yields a fairly uniform specific rate constant over the entire course of the reaction (Fig. 4).

The data for the trichloroacetyl derivatives of

TABLE I

COMPOSITE TABLE OF RELATIVE SUBSTRATE QUALITIES

Amino acid[a]	Carbobenzoxyglycyl derivatives 25°[b] $C_{0.05\ M}$	Q[c]	N-Chloroacetyl derivatives 25°[b] $C_{0.025\ M}$	Q[c]	N-Trichloroacetyl derivatives 25°[b] $C_{0.025\ M}$	Q[c]	Hippuryl derivatives 25°[b] $C_{0.025\ M}$	Q[c]
Phenylalanine	13.0[d]	0.20	2.22	0.16			17.0[e]	0.65
Tyrosine	6.2[f]	0.42	2.00	.17	0.023[g]	0.7	9.96[h]	1.1
Tyrosine			1.44[i]	.24	0.084[j]	0.19	7.51[k]	1.47
Tyrosine			1.61[l]	.22				
Leucine	2.6[m]	1.0	0.348	1.0	0.016	1.0	11.00	1.0
Norvaline			.174	2.0				
Norleucine			.165	2.1	+[n]		8.66	1.28
Isoleucine	0.54[f]	4.8	.087	4.0	0.014	1.14		
Methionine	1.2[o]	2.2	.065	5.3	+[n]		2.32	4.75
Valine			.030	11.4	No act.[p]		1.21	9.1
Alanine	0.04[q]	65	.006	61.5	No act.[p]			
Aspartic acid			No activity[r]					

[a] L-Forms only are substrates. [b] Decimal logarithms were used in the calculation of this value. The lower figure refers to the initial substrate concentration at which measurements were performed. The modified specific rate constant, k', was used for determining the proteolytic coefficients of the chloroacetylated and trichloroacetylated compounds according to the formula: $C = k'/(E)$, where (E) = enzyme concentration. [c] As a result of the evidences of substrate inhibition exhibited by aromatic substrates found here and by Lumry, *et al.*,[11] but lacking in leucine cpds.[11]; these latter were chosen as standards: C, leucine derivative/C, other amino acid derivative = Q. [d] (1) E. L. Smith, *J. Biol. Chem.*, **175**, 39 (1948); (2) E. Elkins-Kaufman and H. Neurath, *ibid.*, **175**, 893 (1948); (3) K. Hofmann and M. Bergmann, *ibid.*, **134**, 225 (1940); (4) H. T. Hanson and E. L. Smith, *ibid.*, **175**, 833 (1948). [e] (d)2. [f] M. A. Stahmann, J. S. Fruton and M. Bergmann, *J. Biol. Chem.*, **164**, 753 (1946). [g] (E) = 0.1295 mg. N/ml. [h] (E) = 0.001295 mg. N/ml. [i] 0.05 M initial substrate concentration. [j] (E) = 0.014 mg. N/ml. [k] (E) = 0.00052 mg. N/ml. [l] 0.045 M initial substrate concentration. [m] Same as (f) plus (d) 4. [n] Very low, but definite activity, (E) = 0.0828 mg. N/ml. [o] C. A. Dekker, S. P. Taylor, Jr., and J. S. Fruton, *J. Biol. Chem.*, **180**, 155 (1949). [p] (E) = 0.0828 mg. N/ml. [q] Same as (f), plus (d)3, and M. Bergmann and J. S. Fruton, *J. Biol. Chem.*, **117**, 189 (1937). [r] (E) = 0.0777 mg. N/ml.

DL-norleucine, DL-methionine, DL-valine and DL-alanine do not permit an extensive kinetic analysis.

Series Comparisons.—Table I presents a comparison of the three series of substrates examined in this work with some carbobenzoxyglycyl derivatives of amino acids.

Of interest within the chloroacetyl series is the equality of the rates observed for the norleucine and norvaline derivatives. Also, the proteolytic quotients of the phenylalanine, isoleucine and alanine members of this series are in excellent agreement with those observed in the carbobenzoxyglycyl group.

In the trichloroacetyl series, the isoleucine compound appears to be as good a substrate as the leucine derivative. Further, its position relative to the norleucine compound is reversed as compared to that observed in the chloroacetyl group.

It is evident from Table I that the trichloroacetyl amino acids are poorer substrates than their chloroacetylated analogs. Two possible explanations for this result may be proffered. The lower rate of hydrolysis of the trichloroacetyl compounds may be due to the factor of steric hindrance as a result of having two hydrogen atoms replaced by two larger chlorine atoms. The magnitude of this factor, if it has reality, is totally unknown. Another explanation may be sought in the electronic aspects of the situation.

It is well known that the trichloromethyl group has a strong meta-directing influence in such reactions as the nitration of the monosubstituted benzene nucleus; whereas, the monochloromethyl group is a strong ortho-, para-directing agent.[12] It might, therefore, be expected that any effect serving to increase the hydrolytic rate by the *chloromethyl* group would be exerted in the opposite direction by the *trichloromethyl* group.

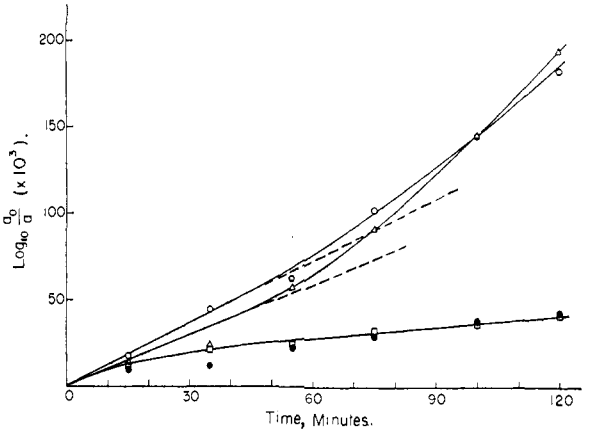


Fig. 4.—Comparison of the rates of hydrolysis of N-trichloroacetyl-L-leucine and N-trichloroacetyl-DL-isoleucine; N-trichloroacetyl-L-leucine: □, experimental rate curve, (E) = 0.0828 mg. N/ml., other conditions standard; ○, modified rate curve; N-trichloroacetyl-DL-isoleucine: ●, experimental rate curve, (E) = 0.0828 mg. N/ml., other conditions standard; △, modified rate curve.

According to the dipositive-bond theory, the chlorine atom of the chloroacetyl group is assigned the function of contributing to the effect tending to make the carbon atom of the susceptible link more positive by drawing negativity toward it, II. With the trichloroacetyl group, the tendency to draw electrons toward the CCl_3 unit by one Cl atom may easily be countered by a negativity contribution from another chlorine atom upon the demand of a carboxyl carbon atom (that of the hydrolyzable bond) which is tending to become extremely positive.

(12) C. R. Noller, "Chemistry of Organic Compounds," 1st ed., W. B. Saunders Co., Philadelphia, Pa., 1951, p. 430.

Smith[13] has drawn attention to the seeming existence of a relationship between the acid strength of the acyl group and the rate of hydrolysis of the acyl amino acid derivative by carboxypeptidase. It was anticipated that the greater the acid strength of the acyl group, the greater would be the rate of hydrolysis as is known to be the case in acid–base hydrolysis. This relationship does have considerable validity; however, trichloroacetic acid is a much stronger acid than chloroacetic acid, which would lead one to expect a greater rate of hydrolysis for the trichloroacetylated amino acids. The results obtained in the present study do not support this concept, in fact, they contradict the notion and demonstrate that something more than acid strength is involved in the hydrolytic process.

The greater activity possessed by hippuryl-DL-leucine and probably hippuryl-DL-norleucine as compared to hippuryl-L-tyrosine was utterly unexpected. As a possible explanation, the question of the presence of a substrate inhibition, dependent upon initial substrate concentration, as regards the tyrosine derivative may be raised in view of the appearance of this phenomenon with chloroacetyltyrosine and with other aromatic amino acid derivatives.[11]

It is noteworthy that similar relative positions were maintained by the hippuryl and chloroacetyl derivatives of leucine, norleucine, methionine and valine. This relative order was also supported from observations on the trichloroacetyl series.

With the exception of the tyrosine compounds and the isoleucine–norleucine relationships, the relative positions between the leucine compounds (standards for the proteolytic quotients) and the other derivatives were, as was expected, reproduced in each series. Further, the hippuryl derivatives appear to be the most potent synthetic substrates for carboxypeptidase thus far produced.

p-Nitrocarbobenzoxyglycyl-L-leucine.—A proteolytic coefficient of 2.0 was obtained for this compound. This indicates that it is only slightly poorer a substrate than carbobenzoxyglycyl-L-leucine (Table I). Though a nitro group in the para position would tend to prevent the secondary peptide nitrogen atom from entering an unfruitful ring A (such as between N_1 and N_2, I), the nitro group itself is a large group with a high dipole moment which could present steric and electrostatic interference to the formation of the proper ES complex.

Acknowledgment.—The author wishes to express his gratitude to Dr. David M. Greenberg for his support of this work.

BERKELEY, CALIF.

(13) E. L. Smith, *Federation Proc.*, **8**, 581 (1949).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, COLUMBIA UNIVERSITY]

Additional Studies of the Properties of Taka Amylase[1]

BY VIRGINIA M. HANRAHAN[2] AND M. L. CALDWELL

RECEIVED FEBRUARY 20, 1953

A study has been made of the stability and certain other properties of highly purified maltase-free and of crystalline maltase-free taka amylase. Calcium ions do not appear to activate taka amylase but do protect it from inactivation under unfavorable conditions. These and other properties of taka amylase are presented and discussed.

Introduction

Quantitative observations of the properties of highly purified or crystalline enzymes are needed to increase our understanding of their nature and of the mechanism of their action and to define the conditions for additional studies. The work reported here deals with such observations for highly purified and for crystalline maltase-free taka amylase.

Experimental

Amylase.—Most of the work reported here was carried out with highly purified maltase-free taka amylase.[3,4] Essential points were repeated with crystalline taka amylase, prepared in general as reported by Fischer and de Montmollin.[5]

Results and Discussion

General Properties of Taka Amylase.—Both types of amylase solution gave essentially the same results. Solutions of the highly purified maltase-free but uncrystallized preparations had the same saccharogenic and amyloclastic activities[6] as solutions of the crystalline amylase.[7] In both cases, the amylase produced 2400 times its weight of maltose equivalents in 30 minutes at 40° from 1% Lintner's soluble potato starch adjusted to 0.01 *M* acetate and *p*H 5.0.[6] In both cases, the solutions were maltase-free and the uncrystallized preparations were found by selective inactivation measurements[3,4,8] to be free from extraneous dextrinase and other contaminating glucosidase activities. Data for this statement are given later.

Several preparations of highly purified maltase-free taka amylase with constant maximum amylase activity were found to be at least 90% homogeneous proteins by electrophoretic measurements.[9] The

(1) The authors wish to thank the Corn Industries Research Foundation for generous grants in aid of this investigation.

(2) The data reported here are taken, in part, from a dissertation submitted by Virginia M. Hanrahan in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Chemistry under the Faculty of Pure Science of Columbia University.

(3) M. L. Caldwell, R. M. Chester, A. H. Doebbeling and G. W. Volz, *J. Biol. Chem.*, **161**, 361 (1945).

(4) Virginia M. Hanrahan, Dissertation, Columbia University, New York, N. Y., 1950.

(5) E. H. Fischer and R. de Montmollin, *Helv. Chim. Acta*, **34**, 1987 (1951).

(6) M. L. Caldwell and S. E. Doebbeling, THIS JOURNAL, **59**, 1835 (1937).

(7) V. M. Hanrahan and M. L. Caldwell, *ibid.*, **75**, 2191 (1953).

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