and 20 g. (0.102 mole of 4-1-octyl)-piperidine in 200 ml. of ether was warmed to reflux for two hours. The amine hydrochloride was removed by filtration and the ether evaporated from the filtrate. White crystals were recovered from the filtrate and recrystallized.

DENTON, TEXAS

[CONTRIBUTION NO. 1666 FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY, CALIFORNIA INSTITUTE OF TECHNOLOGY]

The Enzyme-Inhibitor Dissociation Constants of α-Chymotrypsin and Several Series of Bifunctional Competitive Inhibitors[1]

BY H. T. HUANG AND CARL NIEMANN[2]

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The enzyme-inhibitor dissociation constants of α-chymotrypsin and several series of competitive inhibitors, *i.e.*, benzoic acid, phenylacetic acid, β-phenylpropionic acid, γ-phenylbutyric acid; the corresponding amides; β-indoleacetic acid, β-(β-indole)-propionic acid, γ-(β-indole)-butyric acid; β-(β-indole)-propionamide; and acetanilide, have been determined in aqueous media at 25° and *p*H 7.9 in the presence of a 0.02 *M* tris-(hydroxymethyl)-aminomethane–hydrochloric acid buffer. A comparison of the results obtained in this study with those reported previously has shown that the affinity of α-chymotrypsin for certain negatively charged competitive inhibitors may be determined in part by the nature of the buffer present in the reaction system. The relation between the structure of certain of the above competitive inhibitors has also been considered and attention has been called to the possibility that each of the above inhibitors may combine with the active site of the enzyme in more than one way.

α-Amino acids or simple functional derivatives of these compounds that are specific substrates or competitive inhibitors of α-chymotrypsin may be described by the general formula $R_1CHR_2R_3$, where R_1, R_2 and R_3 are the three prominent structural features of these molecules, *i.e.*, the α-amino or acylamino group, the α-amino acid side chain and the carboxyl group or a functional derivative thereof.[3,4] It has been suggested[4] that the above specific substrates and competitive inhibitors may combine with the enzyme *via* an interaction involving the three groups R_1, R_2 and R_3, and a set of centers, ρ_1, ρ_2 and ρ_3 which are assumed to be a characteristic feature of the catalytically active site of the enzyme and which present a complementary aspect to molecules containing a particular set of R groups disposed in either one of the two possible configurations resulting from the asymmetry of the α-carbon atom present in these molecules. The extent to which any given compound of the general formula $R_1CHR_2R_3$ will be bonded to the active site of the enzyme will primarily depend upon the degree of complementariness originally obtaining between the molecule and the asymmetric catalytic surface, and secondarily, by the ability of both the combining molecule and the active site to alter their respective aspects to improve the closeness of fit during the course of the combining process.

The results of studies conducted in these laboratories on the behavior of specific substrates and competitive inhibitors of α-chymotrypsin have, so far, been consistent with the above view.[4-8] It may therefore be inferred that variations in the nature of disposition of the R groups will be reflected by changes in the corresponding enzyme-inhibitor or enzyme-substrate dissociation constants even in the case of bifunctional compounds where one of the R groups is replaced by a sterically unimportant hydrogen atom. Thus with bifunctional competitive inhibitors of the general formula $R_1(CH_2)_nR_2$, $R_1(CH_2)_nR_3$ and $R_2(CH_2)_nR_3$ it should be possible not only to determine the optimum value of *n* for the best fit to the complementary surface at the active site of the enzyme but also to make a rational estimate of the relative importance of various R groups in the over-all binding process.

In this study we have chosen for examination a group of bifunctional competitive inhibitors $R_2(CH_2)_nR_3$ where R_2 = benzyl or β-indolylmethyl and R_3 = carboxylate or carbamido. When this work was initiated, one member of this group of compounds, *i.e.*, β-phenylpropionic acid, had already been studied[9] although the data were inadequate to establish the nature of the inhibition. The present experiments were all performed at 25° and *p*H 7.9 in aqueous solutions 0.02 *M* with respect to the amine component of a tris-(hydroxymethyl)-aminomethane–hydrochloric acid buffer. In all cases the *p*H of the medium remained within the desired range, *i.e.*, 7.9 ± 0.2 even when the inhibitors containing a carboxylate group were introduced in the form of their sodium salts. Except where noted nicotinyl-L-tryptophanamide was used as the specific substrate with an enzyme concentration corresponding to 0.208 mg. of protein nitrogen per ml. Since in every instance the hydrolytic reaction was limited to within 30% hydrolysis all of the systems investigated can be formulated in terms of equations (1) and (2) where $K_S = (k_2 + k_3)/k_1$ and $K_I = k_5/k_4$.[10] The

$$E_f + S_f \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E_f + P_{1f} + P_{2f} \qquad (1)$$

$$E_f + I_f \underset{k_5}{\overset{k_4}{\rightleftharpoons}} EI \qquad (2)$$

(1) Supported in part by a grant from Eli Lilly and Company.
(2) To whom inquiries regarding this article should be sent.
(3) H. Neurath and G. W. Schwert, *Chem. Revs.*, **46**, 69 (1950).
(4) H. T. Huang and C. Niemann, THIS JOURNAL, **73**, 3223 (1951).
(5) C. Niemann, *Record Chem. Prog.*, **12**, 107 (1951).
(6) H. J. Shine and C. Niemann, THIS JOURNAL, **74**, 97 (1952).
(7) H. T. Huang and C. Niemann, *ibid.*, **74**, 101 (1952).
(8) H. T. Huang, R. J. Foster and C. Niemann, *ibid.*, **74**, 105 (1952).
(9) S. Kaufman and H. Neurath, *J. Biol. Chem.*, **181**, 623 (1949).
(10) For definition of symbols *cf.* H. T. Huang and C. Niemann, THIS JOURNAL, **73**, 1541 (1951).

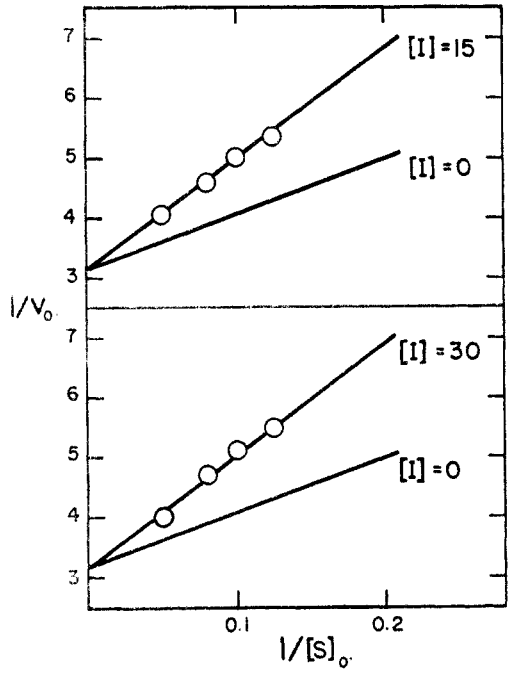

Fig. 1.—Plot of equation (3) for the determination of the K_I values of the first group of inhibitors: v_0 in units of 10^{-3} M per min., $[S]_0$ in units of 10^{-3} M nicotinyl-L-tryptophanamide, [E] equivalent to 0.208 mg. protein-nitrogen per ml.: upper curve, [I] = 15 × 10^{-3} M β-(β-indole)-propionate; lower curve, [I] = 30 × 10^{-3} M β-indoleacetate.

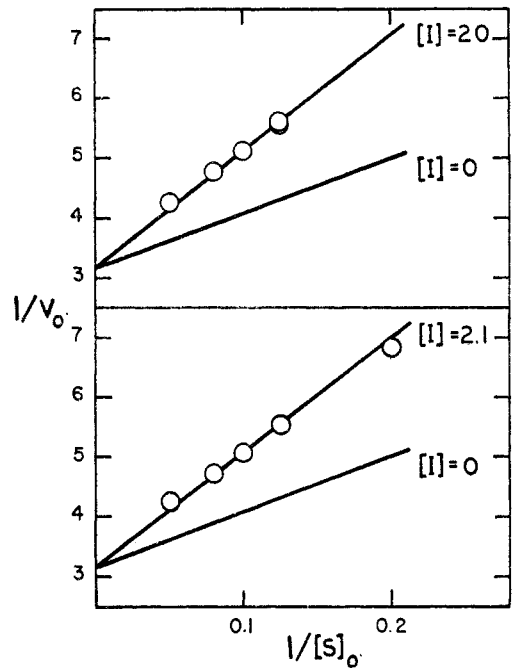

Fig. 2.—Plot of equation (3) for the determination of the K_I values of the second group of inhibitors: v_0 in units of 10^{-3} M per min., $[S]_0$ in units of 10^{-3} M nicotinyl-L-tryptophanamide, [E] equivalent to 0.208 mg. protein-nitrogen per ml.: upper curve, [I] = 20 × 10^{-3} M γ-(β-indole)-butyrate; lower curve, [I] = 2.1 × 10^{-3} M β-(β-indole)-propionamide.

inhibitor concentration used for each individual inhibitor was selected so as to provide a degree of inhibition convenient for the evaluation of K_I from a plot of $1/v_0$ *versus* $1/[S]_0$ based upon equation (3).[7,11]

$$\frac{1}{v} = \frac{K_S}{V}\left(1 + \frac{[I]}{K_I}\right)\frac{1}{[S]} + \frac{1}{V} \qquad (3)$$

The data obtained in the present investigation are summarized in Figs. 1–5 and in Table I. The plots given in Figs. 1–5 leave no doubt that all of the inhibitors studied are competitive inhibitors

TABLE I

ENZYME-INHIBITOR DISSOCIATION CONSTANTS

Inhibitor	H and N K_I[b]	H and N $-\Delta F°$[c]	N and G[a] K_I[d]	N and G[a] $-\Delta F°$[c]
β-Indoleacetate	25	2180		
β-(β-Indole)-propionate	13	2570	2.5	3570
γ-(β-Indole)-butyrate	17	2410	3.6	3350
Benzoate	200	950	42	1880
Phenylacetate	120	1250	42	1880
β-Phenylpropionate	28	2120	5.5	3080
γ-Phenylbutyrate	72	1560	14	2530
β-(β-Indole)-propionamide	1.7	3780		
Benzamide	6.6	2970		
Phenylacetamide	10.2	2720		
β-Phenylpropionamide	6.7	2960		
γ-Phenylbutyramide	7.2	2920		
Acetanilide	10.4	2700		

[a] *Cf.* ref. 12. [b] In units of 10^{-3} M at 25° and *p*H 7.9 in systems 0.02 M in respect to the amine component of a tris-(hydroxymethyl)-aminomethane–hydrochloric acid buffer. [c] In cal. per mole to the nearest 10 cal. [d] In units of 10^{-3} M at 25° and *p*H 7.8 in systems 0.1 M in a phosphate buffer.

(11) H. Lineweaver and D. Burk, THIS JOURNAL, **56**, 658 (1934).

and since K_I is a true equilibrium constant the standard free energy changes for the formation of the various enzyme inhibitor complexes have been calculated. These latter values are given in Table I along with the corresponding enzyme-inhibitor dissociation constants.

Because of the very large difference between the value of K_I which we obtained for β-phenylpropionic acid, *i.e.*, 28 × 10^{-3} M, and the value reported by Kaufman and Neurath,[9] *i.e.*, 4.5 × 10^{-3} M, it was thought desirable to check our value with another specific substrate. Two experiments with $[S]_0$ = 20 × 10^{-3} M acetyl-L-tyrosinamide and [I] = 30 × 10^{-3} M gave a value for K_I of β-phenylpropionic acid, under the conditions previously specified, of approximately 30 × 10^{-3} M. That the difference between our value and that of Kaufman and Neurath[9] must reflect a fundamental difference between the systems we had studied and those investigated by Kaufman and Neurath[9] became evident when, during the final stages of the present investigation, a paper by Neurath and Gladner[12] appeared, in which K_I values were reported for six of the inhibitors listed in Table I, including a revised value for β-phenylpropionic acid, *i.e.*, 5.5 × 10^{-3} M. The values of Neurath and Gladner were determined from $1/v_0$ *versus* $1/[S]_0$ plots using data obtained from aqueous systems at 25° and *p*H 7.8 in which acetyl-L-tyrosinamide was the specific substrate and 0.1 M phosphate the buffer. It is apparent that for every one of the four inhibitors, β-phenylpropionic acid, γ-

(12) H. Neurath and J. A. Gladner, *J. Biol. Chem.*, **188**, 407 (1951).

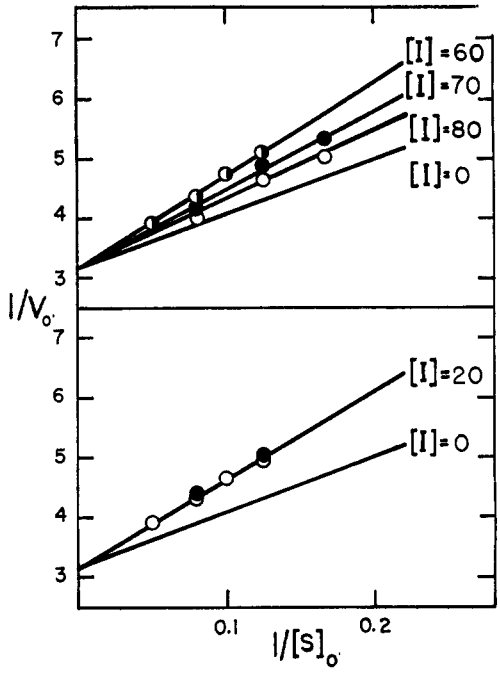

Fig. 3.—Plot of equation (3) for the determination of the K_I values of the third group of inhibitors: v_0 in units of 10^{-3} M per min., $[S]_0$ in units of 10^{-3} M nicotinyl-L-tryptophanamide, [E] equivalent to 0.208 mg. protein-nitrogen per ml.: upper curve, ◑, [I] = 60 × 10^{-3} M γ-phenylbutyrate; ●, [I] = 70 × 10^{-3} M phenylacetate; ○, [I] = 80 × 10^{-3} M benzoate; lower curve, [I] = 20 × 10^{-3} M β-phenylpropionate.

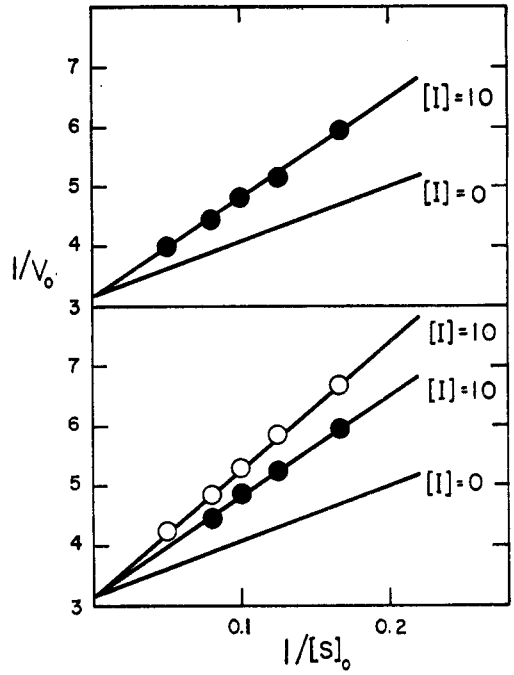

Fig. 4.—Plot of equation (3) for the determination of the K_I values of the fourth group of inhibitors: v_0 in units of 10^{-3} M per min., $[S]_0$ in units of 10^{-3} M nicotinyl-L-tryptophanamide, [E] equivalent to 0.208 mg. protein-nitrogen per ml.: upper curve, [I] = 10 × 10^{-3} M acetanilide; lower curve, ○, [I] = 10 × 10^{-3} M benzamide; ●, [I] = 10 × 10^{-3} M phenylacetamide.

phenylbutyric acid, β-(β-indole)-propionic acid and γ-(β-indole)-butyric acid, where it has been possible for us to evaluate K_I at near optimum inhibitor concentration,[7] our values, *i.e.*, 28, 72, 13 and 17 × 10^{-3} M are very nearly five times those given by Neurath and Gladner,[12] *i.e.*, 5.5, 14, 2.5 and 3.6 × 10^{-3} M.[13] The difference between our values for competitive inhibitors of the type $R_2(CH_2)_nR_3$, where R_2 = benzyl or β-indolymethyl and R_3 = carboxylate and those of Neurath and Gladner[12] corresponds to a decrease in $-\Delta F°$, in passing from our reaction system to that of Neurath and Gladner,[12] of approximately 1000 cal. per mole, which is a large fraction of the $-\Delta F°$ values encountered for the formation of the enzyme-inhibitor complexes of all competitive inhibitors that have so far been studied in detail.[4,6-10,12,14-18]

The relatively good agreement between the K_S value of acetyl-L-tyrosinamide determined in systems 0.02 M in the amine component of a tris-(hydroxymethyl)-aminomethane–hydrochloric acid buffer and that obtained in systems 0.1 M in a phosphate buffer[14] gives no indication that this value is markedly sensitive to the above changes in ionic strength or ion species. A further indication of the

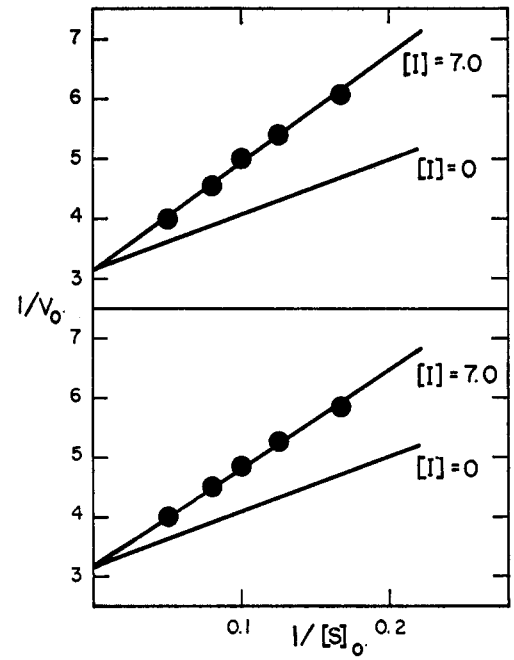

Fig. 5.—Plot of equation (3) for the determination of the K_I values of a fifth group of inhibitors: v_0 in units of 10^{-3} M per min., $[S]_0$ in units of 10^{-3} M nicotinyl-L-tryptophanamide, [E] equivalent to 0.208 mg. protein-nitrogen per ml.: upper curve, [I] = 7 × 10^{-3} M β-phenylpropionamide; lower curve, [I] = 7 × 10^{-3} M γ-phenylbutyramide.

(13) It is felt that our values for benzoic acid and phenylacetic acid are not sufficiently accurate for quantitative comparisons, since the measured increases in the slopes of the $1/v_0$ *versus* $1/[S]_0$ plots are very small in these cases, *cf.* Fig. 3.

(14) D. W. Thomas, R. V. MacAllister and C. Niemann, THIS JOURNAL, **73**, 1548 (1951).

(15) H. T. Huang and C. Niemann, *ibid.*, **73**, 1555 (1951).

(16) H. T. Huang and C. Niemann, *ibid.*, **73**, 3228 (1951).

(17) H. T. Huang, R. V. MacAllister, D. W. Thomas and C. Niemann, *ibid.*, **73**, 3231 (1951).

(18) H. T. Huang and C. Niemann, *ibid.*, **73**, 4039 (1951).

lack of sensitivity of the K_S values of certain specific substrates to changes in ionic strength or ion species is to be found in the excellent agreement between the K_S value of glycyl-L-tyrosinamide determined at *p*H 7.8 in a tris-(hydroxymethyl)-aminomethane–hydrochloric acid buffer of unspecified concentration, *i.e.*, 120×10^{-3} *M*,[19] and the value obtained at *p*H 7.8 in a 0.1 *M* phosphate buffer, *i.e.*, 122×10^{-3} *M*.[20] However, it is conceivable that a change in ionic strength could have a far more pronounced effect upon the K_I values of carboxylic acids. An increase in ionic strength could cause a decrease in the activity coefficient of the inhibitor anion and hence lead to an increase in the K_I value. On the other hand if, as it seems likely, a negative charge is situated in the vicinity of the catalytically active site,[3] the approach of a negatively charged inhibitor ion would be enhanced by this increase in ionic strength, thus causing a decrease in the K_I value. While the balance of these two opposing effects may be in favor of combination, it is difficult to see from these considerations how an increase in ionic strength, within the limits involved, can be solely responsible for the difference noted in the two buffer systems. Therefore, it appears that the difference in the two sets of K_I values, for the four bifunctional anionic competitive inhibitors being compared, may be due to a specific ion effect that is operative in one or both of the buffer systems and which profoundly influences the nature of the reaction occurring between the enzyme and certain negatively charged competitive inhibitors of the hydrolytic reaction.

Despite the fivefold discrepancy in the two sets of K_I values for the carboxylic acids listed in Table I, it is clear that our results are in excellent agreement with those of Neurath and Gladner[12] with respect to the importance of the degree of separation of the R_2 and R_3 groups in determining the affinity of the enzyme for inhibitors of the general formula $R_2(CH_2)_nR_3$. It is interesting to note that the K_I value of β-(β-indole)-propionamide, 1.7×10^{-3} *M*, is actually less than both the K_S value of acetyl-L-tryptophanamide, 5.3×10^{-3} *M*, and the K_I value of acetyl-D-tryptophanamide, 2.7×10^{-3} *M*.[10] Similarly, the K_I value of β-phenylpropionamide, 6.7×10^{-3} *M*, is less than both the K_S value of acetyl-L-phenylalaninamide, 34×10^{-3} *M*,[8] and the K_I value of acetyl-D-phenylalaninamide, 14×10^{-3} *M*.[8] These results are in accord with the idea, expressed previously,[3,7] that the strain present in an intermediate complex derived from a trifunctional interaction, *i.e.*, R_1–ρ_1, R_2–ρ_2 and R_3–ρ_3, is greater than that in one involving a bifunctional interaction, *e.g.*, R_2–ρ_2 and R_3–ρ_3. They also indicate that the acetamido and carbamido functions are inherently groups of low affinity for the enzymatic site, in contrast to the β-indolylmethyl and benzyl groups, which are apparently primarily responsible for the favorable binding free energy in the interaction between the enzyme and the above inhibitor.

The value of K_I for β-(β-indole)-propionate, 13×10^{-3} *M*, lies between the K_I value of acetyl-L-tryptophanate, 17.5×10^{-3} *M*,[10] and that of acetyl-D-tryptophanate, 4.8×10^{-3} *M*,[4] while the K_I value of β-(β-indole)-propionamide, 1.7×10^{-3} *M*, is less than both the K_S value of acetyl-L-tryptophanamide, 5.3×10^{-3} *M*,[10] and the K_I value of acetyl-D-tryptophanamide, 2.7×10^{-3} *M*.[10] These results suggest that when R_3 = carboxylate this group has very little affinity for the corresponding ρ_3 center in systems containing a 0.02 *M* tris-(hydroxymethyl)-aminomethane–hydrochloric acid buffer. Thus, when R_3 = carboxylate, in spite of the expectation that some strain will be created by the interaction of the three R groups of acetyl-D-tryptophanate with the corresponding ρ centers of the catalytically active site, the attractive forces, principally due to the interaction of R_1 and ρ_1 and R_2 and ρ_2, result in a more favorable $-\Delta F^\circ$ than in the case of β-(β-indole)-propionate, where only one interaction, *i.e.*, between R_2 and ρ_2, can effectively contribute to the binding energy. However, when R_3 is a carbamido group the interaction of R_3 and ρ_3 clearly results in an attraction and this added increment contributes more effectively to the binding energy of the bifunctional β-(β-indole)-propionamide, *i.e.*, 3780 cal., than to that of the trifunctional acetyl-D-tryptophanamide, *i.e.*, 3500 cal.,[10] and actually results in a greater $-\Delta F^\circ$ for the former compound.

With the 0.02 *M*-tris-(hydroxymethyl)-aminomethane–hydrochloric acid buffer the difference in $-\Delta F^\circ$ between the two pairs β-(β-indole)-propionamide and β-(β-indole)-propionate and β-phenylpropionamide and β-phenylpropionate is 1600 and 840 cal., respectively. However, if the $-\Delta F^\circ$ values of Neurath and Gladner,[12] which were determined in systems containing a 0.1 *M* phosphate buffer, are used for the above carboxylate ions the difference, in $-\Delta F^\circ$, is 210 and −120 cal., respectively. Thus it appears that the 0.1 *M* phosphate buffer is capable of increasing the affinity of the enzyme for these carboxylate ions to the point where the difference observed in the first buffer system, is, for all practical purposes, abolished. In this connection it is of interest to note that Neurath and Gladner[12] found that in the 0.1 *M* phosphate buffer the K_I values of β-phenylpropionate and β-phenoxyethanol were 5.5 and 5.8×10^{-3} *M*, respectively.

In the series $R_2(CH_2)_nR_3$ where R_2 = benzyl and R_3 = carbamido and n = 0, 1 and 2, it is seen that the most effective inhibitor is obtained when n = 1, and that the affinity is decreased when n is changed to 0 or 2. The decrease in affinity is, however, much less pronounced than that observed for the same changes where R_2 = benzyl and R_3 = carboxylate. Furthermore, benzamide, which can be regarded either as a member of the above series with $n = -1$, *i.e.*, as a R_2 group, or as a benzamido derivative, *i.e.*, as a R_1 group, has about the same K_I value as that of β-phenylpropionamide. Apart from further emphasizing the relatively low affinity of the carbamido group for ρ_3, these considerations suggest that our interpretation of the mode of combination of α-chymotrypsin with competitive inhibitors of this enzyme may be grossly oversimplified

(19) D. C. Doherty and F. Vaslow, THIS JOURNAL, **74**, 931 (1952).
(20) S. Kaufman, H. Neurath and G. W. Schwert, *J. Biol. Chem.*, **177**, 793 (1949).

particularly when applied to bi- and monofunctional inhibitors. If it is assumed that the benzamido group in benzamide can interact with the center ρ_2, it is also necessary to assume that it can interact with the center ρ_1.[4,7] While it is conceivable that benzamide may interact with one center more readily than with the other, both of these intermediate complexes would block combination of the catalytically active site of the enzyme with trifunctional specific substrates such as nicotinyl-L-tryptophanamide and acetyl-L-tyrosinamide. Therefore the experimental value of K_I for benzamide could be a function of two separate dissociation constants, one for each of the above two intermediate complexes. The situation may be even more complicated since the existence of a third complex based upon simultaneous interaction of two molecules of benzamide with the centers ρ_1 and ρ_2 cannot be entirely excluded. Thus the relatively low value of K_I for benzamide may reflect an enhanced probability of combination arising from a multiplicity of modes of combination rather than a high affinity in any particular mode. At present it is not possible to evaluate quantitatively the relative contributions of each of the three types of intermediate complexes that may be formed.

While it may be fortuitous that the K_I value of acetanilide, 10.4×10^{-3} M, is practically identical with that of phenylacetamide, 10.2×10^{-3} M, it does appear that with these two isomeric competitive inhibitors a transposition of the $-CH_2-$ and $-NH-$ groups present in their side chains is without demonstrable effect upon the over-all binding process. This indicates that with these two compounds the principal factor in the combination process is the size and shape of the molecule, and hence a van der Waals interaction.

The data obtained during the course of this investigation have pointed out the existence of two fundamental aspects of certain enzyme-inhibitor and enzyme-substrate interactions involving α-chymotrypsin, *viz.*, (a) the fact that the affinity of the enzyme for certain anionic competitive inhibitors may be profoundly influenced by the nature of the buffer present in the reaction system, and (b) that several different modes of combination may be involved in the interaction of this enzyme with a given specific substrate or competitive inhibitor. Since both of these issues are important for a correct understanding of available kinetic data it is hoped that studies now in progress will expand our knowledge along these lines.

Experimental[21,22]

Competitive Inhibitors.—β-Indoleacetic acid, m.p. 167–168°; β-(β-indole)-propionic acid, m.p. 133–134°; γ-(β-indole)-butyric acid, m.p. 123–124°; benzoic acid, m.p. 122–123°; phenylacetic acid, m.p. 76–77°; β-phenylpropionic acid, m.p. 48–49°; γ-phenylbutyric acid, m.p. 50–51°; acetanilide, m.p. 113–114°; and benzamide, m.p. 129–130°, were Eastman Kodak Co. products which were recrystallized at least twice from appropriate solvents. Phenylacetamide, m.p. 157–158°; β-phenylpropionamide, m.p. 105–106°, and γ-phenylbutyramide, m.p. 84–85°, were prepared from the corresponding acids by ammonolysis of the respective acid chlorides. Ammonolysis of 3 g. of crude methyl β-(β-indole)-propionate, prepared by the reaction of methanolic hydrogen chloride with the corresponding acid, gave 1.5 g. of β-(β-indole)-propionamide, fine stunted needles, m.p. 204–205°.

Anal. Calcd. for $C_{11}H_{12}ON_2$ (188): C, 70.3; H, 6.4; N, 14.9. Found: C, 70.3; H, 6.2; N, 14.9.

Enzyme Experiments.—The methods used were identical with those described previously. The preparation of the principal specific substrate, *i.e.*, nicotinyl-L-tryptophanamide for which $K_S = 2.7 \times 10^{-3}$ M,[10] has also been described.[23] The enzyme preparation was an Armour product, lot no. 90402, and it will be noted that the specific enzyme concentrations E'_S and E'_I for all experiments were such as to satisfy zone A conditions.[10,24,25]

(21) Microanalyses by Dr. A. Elek.

(22) All melting points are corrected.

(23) B. M. Iselin, H. T. Huang, R. V. MacAllister and C. Niemann, THIS JOURNAL, **72**, 1729 (1950).

(24) O. H. Straus and A. Goldstein, *J. Gen. Physiol.*, **26**, 559 (1943).

(25) A. Goldstein, *ibid.*, **27**, 529 (1944).

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[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT OF FORDHAM UNIVERSITY]

Effect of Methyl Substitution on the Decarboxylation of Picolinic Acids

BY NELSON H. CANTWELL AND ELLIS V. BROWN

RECEIVED NOVEMBER 12, 1951

In an attempt to elucidate the decarboxylation mechanism of picolinic acid, the rates of decarboxylation of picolinic acid itself, four monomethyl derivatives and one dimethyl derivative were studied. The observed reaction rates were found to be first order in all cases. The E and A values of the Arrhenius equation and the $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ values of the absolute reaction rate theory were then determined. On the basis of the data obtained, mechanisms for the decarboxylation of picolinic acid and the effect of methyl substitution on the reaction are proposed.

In the process of investigating the mechanism of the Hammick reaction,[1] the necessity of having some knowledge concerning the mechanism of decarboxylation of the acid involved in the reaction became immediately apparent. Picolinic acid, one of the four acids which have been shown to undergo the Hammick coupling upon decarboxylation, was chosen for study. It was thought advisable to determine the rate of the uncatalyzed decarboxylation of picolinic acid and some of its methyl derivatives which might then help to elucidate this process. Uncatalyzed reactions were selected because a report[2] on the rate of breakdown of picolinic acid in various solvents did not agree with the rate as observed during the Hammick reaction.

The question then arose as to what mechanism might be postulated to account for the ease with which picolinic acids and acids similar in structure decarboxylate. One can postulate three reactive

(1) P. Dyson and D. L. Hammick, *J. Chem. Soc.*, 1725 (1937); 809 (1939); 173 (1949); 659 (1949).

(2) H. Schenkel and A. Klein, *Helv. Chim. Acta*, **28**, 1211 (1945).