suspended in 100 ml. of chloroform, the slurry added to 500 ml. of chloroform previously saturated with dry gaseous ammonia, the precipitated ammonium chloride removed by filtration, the filtrate evaporated to dryness *in vacuo* and the residue dried *in vacuo* over phosphorus pentoxide to give 29.6 g. of crude L-tyrosine ethyl ester; yield 59.4%. Acetylation of the crude ester with acetyl chloride, under Schotten–Bauman conditions, gave 29.0 g. (83%) of acetyl-L-tyrosine ethyl ester. This product was dissolved in absolute ethanol and the solution added to 3.8 g. of hydrazine in the same solvent. The reaction mixture was heated under refluxing conditions for 2 hr., cooled, the precipitated product collected, recrystallized twice from absolute methanol and dried *in vacuo* over phosphorus pentoxide to give 23.4 g. (83%) of $\alpha$-N-acetyl-L-tyrosinhydrazide, m.p. 227–223°, $[\alpha]^{25.2}$D 40.8 ± 0.6° ($c$ 2.02%, in Methyl Cellosolve).

*Anal.* Calcd. for $C_{11}H_{15}O_3N_3$ (237): C, 55.7; H, 6.4; N, 17.7. Found: C, 55.6; H, 6.5; N, 17.8.

**Enzyme Solutions.**—An Armour preparation, lot no. 00592, was used throughout. The stock solutions were prepared as before[5] except that 50 mg. of the enzyme preparation was dissolved in 5.0 ml. of distilled water. One ml. of the enzyme stock solution diluted 1:10 led to a final concentration of 1 mg. per ml. or, when based upon a nitrogen content of 14.5%, to a concentration of 0.145 mg. of protein-nitrogen per ml. or $4.55 \times 10^{-5}$ $M$.[2b]

**Buffer Solution.**—A THAM–HCl buffer stock solution, 0.2 $M$ in the amine component, was prepared as before.[5]

**Enzymatic Reaction Systems and their Analysis.**—The reaction systems were established essentially as described previously.[5] The analyses were conducted under conditions

where the final concentration of aldehyde was $2.684 \times 10^{-2}$ $M$ and the hydrochloric acid concentration 0.167 $N$. In practice an acidic aldehyde reagent was prepared immediately before use by mixing equal volumes of the acid and aldehyde reagent solutions, *vide ante*. A 2.0-ml. aliquot of the acidic aldehyde reagent was introduced into a series of 10.0-ml. G.S. volumetric flasks and sufficient distilled water added to each flask to bring the volume to 9.0 ml. At selected time intervals, usually two minutes, a 1.0-ml. aliquot of the reaction mixture was transferred to a flask, the solution equilibrated at 25.0 ± 0.1° for exactly 20 minutes whereupon the optical density was determined at 455 m$\mu$ as indicated above except that in this instance the blank contained all of the components of the reaction and analyses systems other than the reaction products. The blank was prepared by diluting the acidic aldehyde reagent with the buffered specific substrate solution[5] and then adding the enzyme solution. When it became evident that the optical density would soon exceed a value of 1.1, larger volumetric flasks were substituted for the 10.0-ml. flasks used initially and the optical density corrected and recorded as its equivalent in a 10.0-ml. flask. Whenever this latter practice was followed, care was taken to maintain the final acid and aldehyde concentrations at a constant value by proportionally increasing the amount of acidic aldehyde reagent added to each flask, *i.e.*, 5.0 ml. to a 25.0 ml. and 10.0 ml. to a 50.0 ml. flask, and proportionally diluting the acidic aldehyde reagent before introducing the 1.0-ml. aliquot obtained from the reaction system. Further experimental details are summarized in Table I.

PASADENA, CALIFORNIA

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# The Apparent Ionization Constants of a Series of Phenylalanine Derivatives[1]

BY HAROLD R. ALMOND, JR., RICHARD J. KERR AND CARL NIEMANN[2]

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The apparent ionization constants of the $\alpha$-ammonium groups present in DL-phenylalanine and its monoprotonated amide, thioamide, amidoxime, hydrazide, methyl ester and hydroxamide have been determined in aqueous solutions at 25.0 ± 0.1° and 0.05, 0.10 and 0.20 $M$ in sodium chloride. The values of $pK^0{}_A(\text{NH}_3-)$ were observed to increase from 6.78 ± 0.03 to 9.15 ± 0.01 in the order $-\text{CONHOH} < -\text{CO}_2\text{CH}_3 \leq -\text{CONHNH}_2 \doteq -\text{C(NOH)NH}_2 < -\text{CSNH}_2 < -\text{CONH}_2 \ll -\text{CO}_2{}^-$. Where comparison was possible the phenylalanine derivatives were found to have $pK'{}_A(\text{NH}_3+)$ values that were 0.59 ± 0.04 of a $pK$ unit lower than those of the corresponding glycine derivatives. The infrared spectra of all of the phenylalanine derivatives were determined for the solid in solid potassium bromide.

The use of amino acid derivatives, containing an $\alpha$-amino or $\alpha$-ammonium group, and an aromatic side chain, as specific substrates of $\alpha$-chymotrypsin[3–7] has created a demand for knowledge of the apparent ionization constants of the $\alpha$-ammonium groups present in these compounds.[7] In addition such data were required for an evaluation of the influence of the aromatic nucleus and the adjacent carboxyl function upon the above ionization constants.

Among the pertinent data that were available at the time this study was initiated were the $pK'{}_A$ values of the $\alpha$-ammonium group of glycine, 9.72[8]; alanine, 9.72[8]; phenylalanine, 9.12,[9] 9.13,[8]

9.15[10]; $o$-, $m$- and $p$-fluorophenylalanine, 9.01,[9] 8.98[9] and 9.05[9]; $o$-, $m$- and $p$-chlorophenylalanine, 8.94,[10] 8.91[10] and 8.96[10]; $p$-sulfamylphenylalanine, 8.64[10,11]; tyrosine, 9.11[8,11,12]; tryptophan, 9.39[8]; glycine methyl ester, 7.66[8]; glycine ethyl ester, 7.73[8]; alanine methyl ester, 7.80[8]; leucine methyl ester, 7.63[8]; methyl $\alpha$-amino-$n$-butyrate, 7.71[8]; tyrosinhydroxamide, 7.05[5,11]; glycinamide, 7.93[8]; tryptophanamide, 7.51[14]; and glycinhydrazide, 7.69.[11,15] In order to provide a more systematic set of data it was decided to examine a series of phenylalanine derivatives in which the nature

tides as Ions and Dipolar Ions." Reinhold Publ. Corp., New York, N. Y., 1943.

(9) E. L. Bennett and C. Niemann, THIS JOURNAL, **72**, 1804 (1950).

(10) J. C. Nevenzel, W. E. Shelberg and C. Niemann, *ibid.*, **71**, 3024 (1949).

(11) This value refers to the macroscopic ionization constant.

(12) In a recent communication Edsall, Martin and Hollingworth[13] report a value of 9.11 for the macroscopic ionization constant. They also give values for the microscopic constants relative to the ionization of the $\alpha$-ammonium group for the case where the phenolic hydroxyl group is ionized, 9.70, and where it is not, 9.28.

(13) J. T. Edsall, R. B. Martin and B. R. Hollingworth, *Proc. Natl. Acad. Sci.*, **44**, 505 (1958).

(14) H. T. Huang and C. Niemann, THIS JOURNAL, **73**, 3223 (1951).

(15) C. R. Lindegren and C. Niemann, *ibid.*, **71**, 1504 (1949).

(1) Supported in part by a grant from the National Institutes of Health, Public Health Service.

(2) To whom inquiries regarding this article should be sent.

(3) E. F. Jansen, M. D. Fellows-Nutting, R. Jang and A. K. Balls, *J. Biol. Chem.*, **179**, 189 (1949); **185**, 209 (1950).

(4) E. F. Jansen, A. L. Curl and A. K. Balls, *ibid.*, **189**, 671 (1951).

(5) R. J. Foster, R. R. Jennings and C. Niemann, THIS JOURNAL, **76**, 3142 (1954).

(6) R. J. Foster and C. Niemann, *ibid.*, **77**, 1886 (1955).

(7) R. Lutwack, H. F. Mower and C. Niemann, *ibid.*, **79**, 2179, 5690 (1957).

(8) E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids and Pep-

of the carboxyl function was varied so as to include a number of representative types. The compounds chosen for study were phenylalanine, its methyl ester, hydroxamide, amidoxime,[16] amide, thioamide[16] and hydrazide. All of the above compounds, present as their hydrochlorides in aqueous solutions at $25.0 \pm 0.1°$ and 0.05, 0.10 or 0.20 $M$ in sodium chloride, were titrated with aqueous sodium hydroxide using an automatic recording titrator.[17]

Fenwick[18] has assumed that a titration curve, i.e., a plot of e.m.f. or $E$, vs. volume of added titrant, $V$, is the region of the equivalence point may be represented by equation 1 and the point of inflection, $V_i$ coincident with the equivalence point

$$E = aV^3 + bV^2 + cV + d \qquad (1)$$

by equation 2. Thus, the solution of a set of simultaneous equations based upon equation 1

$$d^2E/dV^2 = 6aV_i + 2b = 0 \qquad (2)$$

and data obtained from the plot in the region of the equivalence point led to values of the coefficients $a$ and $b$ and by substitution of these values in equation 2 to a value of $V_i$.[18]

Since the titration curve for a monobasic acid also possesses an inflection point at the half-equivalence point, where $pH = pK'_A$, a procedure similar to that developed by Fenwick[18] may be used to evaluate $pK'_A$. In the case at hand a third degree orthogonal polynomial was fitted to the trace of the titration curve by the method of least squares using nine equidistant points, i.e., $s = 0,1,2, \ldots 8$, along the $V$-axis so spaced as to encompass an extent of neutralization of ca. 70%.[19] In practice the orthogonal polynomials $P_m$, with coefficients $C_m$, given in equation 3 were developed from the table given by Milne[20] and

$P_{0(s)} = 1$

$P_{1(s)} = 4 - (s)$

$P_{2(s)} = 28-21(s) + 3(s)(s - 1)$

$P_{3(s)} = 14-21(s) + 7.5(s)(s - 1) - 0.833(s)(s - 1)$
$\qquad\qquad (s - 2) \qquad (3)$

$Y = C'_0 + C'_1(s) + C'_2(s)(s - 1) + C'_3(s)(s - 1)$
$\qquad\qquad (s - 2) \qquad (4)$

transformed into equation 4 where $C'_0 = C_0 + 4C_1 + 28C_2 + 14C_3$, $C'_1 = -C_1 - 21C_2 - 21C_3$, $C'_2 = 3C_2 + 7.5C_3$ and $C'_3 = -0.833C_3$. Dif-

$$d^2\bar{Y}/ds^2 = 2C'_3 + 6C'_3(s - 1) \qquad (5)$$

$$s = 1 - (C'_2/3C'_3) = 4 - 1.2 (C_2/C_3) \qquad (6)$$

ferentiation of equation 4 leads to equation 5 and to equation 6 for the condition $d^2\bar{Y}/d\ s^2 = 0$.

Since $C_m = c_m/S_m$, where $S_m = \sum_{s=0}^{8} [P_m(s)]^2$ and $c_m = \sum_{s=0}^{8} P_m(s) \cdot f(s)$, $s = 4 + 0.4286(c_2/c_3)$. The coefficients $c_2$ and $c_3$ are readily calculated from the tabulated values of $P_m$[20] and the observed values

of $pH$. i.e., $f(s)$. The value of $pH$ at the inflection point, $pH_i$, is then determined by interpolation using the value of $s$ obtained as above. If by definition, $K'_A = (H^+)$ [base]/[acid], where parentheses and brackets denote activity and concentration, respectively; the total amount of salt initially added, [NaCl], $= n$; the total amount of acid, $\Sigma R$, $= r$; the concentration of added titrant in solution $= B$ and $w = B + [H^+] - [OH^-]$ and if (a) the system is chosen so that $n >> r >> [H^+] - [OH^-]$ and (b) the normality of the added titrant and the total volume of solution are sufficiently great so that $B = $ constant x $V$ it follows that $dK'_A/dB \doteq 0$ and $B \doteq w$. For a monobasic acid, $HR^+ \rightleftarrows H^+ + R$, a treatment similar to that of Auerbach and Smolczyk[21] gives the exact relation $pK'_A = pH - \log (w/(r-w))$. Setting $d^2 pH/dw^2 = 0$ gives $W_i = 0.5r$ or $pK'_A = pH_i$.[22]

All of the primary data, i.e., the recorder traces, were evaluated as described above and the results obtained are summarized in Table I. The derivatives listed in this table are arranged in order of increasing acidity of the $\alpha$-ammonium group, or decreasing basicity of the $\alpha$-amino group.

Consideration of the $pK'_A$ values of the $\alpha$-ammonium group of glycine dipolar ion, 9.72,[8] and of phenylalanine dipolar ion, 9.15,[10] would lead one to anticipate lower $pK'_A$ values for the $\alpha$-ammonium group of carboxyl derivatives of a monoprotonated phenylalanine than those of the corresponding glycine derivatives, particularly since the $pK'_A$ value of $\beta$-phenylethylammonium ion, 9.83,[23] is markedly lower than that of methylammonium ion, 10.64,[23] ethylammonium ion, 10.67,[23] or $n$-propylammonium ion, 10.58.[23,24] It will be seen from the data given in Table II that the expected behavior is observed wherever a comparison can be made. It also appears that the difference in the $pK'_A$ values observed for the members of each pair is substantially independent of the nature of the carboxyl function present in a given pair. Thus, one may predict that the $pK'_A$ value of the $\alpha$-ammonium group in a protonated phenylalanine derivative containing this group will be $0.59 \pm 0.04$ of a $pK$ unit lower than that of the corresponding glycine derivative.[25]

In an earlier communication[7] it was estimated, on the basis of a $pK'_A$ value of 7.3 for the $\alpha$-ammonium group of monoprotonated phenylalanine methyl ester, and the near equivalence of the macroscopic $pK'_A$ values of the $\alpha$-ammonium groups of phenylalanine and tyrosine, 9.15 and 9.11, that the $pK'_A$ value of the $\alpha$-ammonium group of monoproto-

(16) P. E. Peterson and C. Niemann, THIS JOURNAL, 79, 1389 (1957).

(17) J. B. Neilands and M. D. Cannon, Anal. Chem., 27, 29 (1955).

(18) F. Fenwick, Ind. Eng. Chem., Anal. Ed., 4, 144 (1932).

(19) The use of 17 instead of 9 points led to a difference in $pK'_A$ values of less than $\pm 0.003$ of a $pK$ unit.

(20) W. E. Milne, "Numerical Calculus," Princeton University Press, Princeton, N. J., 1949, pp. 265-271, 375-381.

(21) F. Auerbach and E. Smolczyk, Z. physik. Chem., 110, 65 (1924).

(22) For a dibasic acid, $H_2R^+ \rightleftarrows H^+ + HR$; $HR \rightleftarrows H^+ + R^-$, it can be shown that $d^2 pH/dw^2 = 0$ has solutions at $pH_i = (pK'_{A1} + pK'_{A2})/2$, $pK'_{A1} = pH_i - \log [(1 + 8K'_{A2}/h_i)/(1 - K'_{A2}/h_i)]$ and $pK'_{A2} = pH_i - \log [(1 - h_i/K'_{A1})/(1 + 8h_i/K'_{A1})]$ where $h = (H^+)$.

(23) H. C. Brown, D. H. McDaniel and O. Häfliger, in E. A. Braude and F. C. Nachod, "Determination of Organic Structures by Physical Methods," Academic Press, Inc., New York, N. Y., 1955.

(24) In the series $C_6H_5(CH_2)_nNH_3$ and for values of $n = 0$ to 5 the $pK'_A$ values are 4.58, 9.37, 9.83, 10.20, 10.39 and 10.49, respectively.[23] i.e., the influence of the phenyl group is still evident at $n = 5$.

(25) When it is recalled that the difference between the $pK'_A$ value of methylammonium ion and that of $\beta$-phenylethylammonium ion is 0.81 of a $pK$ unit it will be obvious that this prediction cannot be extrapolated to the extreme case where the carboxyl function is replaced by hydrogen.

TABLE I

APPARENT IONIZATION CONSTANTS OF A SERIES OF PHENYLALANINE DERIVATIVES[a]

| Derivative | [NaCl], $M$ | $pK'_A(NH_3^+)$ Separate values[b] | $pK'_A(NH_3^+)$ Average values[c] | Extrapolated values[d] |
|---|---|---|---|---|
| DL-Phenylalanine | 0.05 | 9.18 | | |
| | .10 | 9.19 | | |
| | .20 | 9.17 ± .01[e] | 9.18 ± 0.01 | |
| DL-Phenylalaninamide | .05 | 7.30 | | |
| | .10 | 7.31 | | |
| | .20 | 7.35 ± .01[e] | 7.33 ± .02 | 7.22 ± 0.01 |
| DL-Phenylalaninthioamide | .05 | 7.23 | | |
| | .10 | 7.21 | | |
| | .20 | 7.27 ± .03[f] | 7.26 ± .03 | 7.15 ± .02 |
| DL-Phenylalaninamidoxime | .05 | 7.15 | | |
| | .10 | 7.16 | | |
| | .20 | 7.18 ± .01[e] | 7.17 ± .02[m] | 7.06 ± .01[m] |
| DL-Phenylalaninhydrazide | .05 | 7.12 ± .02[g] | | |
| | .10 | 7.15 ± .01[g] | | |
| | .20 | 7.19 ± .02[h] | 7.16 ± .04 | 7.06 ± .01 |
| DL-Phenylalanine methyl ester | .05 | 7.06 ± .01[g] | | |
| | .10 | 7.06 ± .03[g] | | |
| | .20 | 7.14 ± .03[i] | 7.11 ± .04 | 7.00 ± .03 |
| DL-Phenylalaninhydroxamide[i,k,l] | .05 | 6.83 ± .01[g] | | |
| | .10 | 6.88 ± .02[g] | | |
| | .20 | 6.92 ± .04[h] | 6.89 ± .04[m] | 6.78 ± .03[m] |

[a] In aqueous solutions at 25.0 ± 0.1°.  [b] Value may be that of a single determination or the mean of several, in which case the standard deviation is given.  [c] Mean of all determinations from 0.05 to 0.20 $M$ NaCl.  [d] Value for $\mu = 0$, i.e., $pK_A^0 _{NH_3^+}$, based upon the simple Debye–Hückel relation and an assumed effective collision diameter of 6 Å.[8]  [e] Mean of three determinations.  [f] Mean of four determinations.  [g] Mean of two determinations.  [h] Mean of five determinations.  [i] Mean of seven determinations.  [j] All values corrected for overlap of ionization of ammonium and hydroxamide groups.[22]  [k] $pK'_A$ values for ionization of hydroxamide group: 0.05 $M$ NaCl, 9.14; 0.10 $M$ NaCl, 9.12; 0.20 $M$ NaCl, 9.10 ± 0.01[e]; extrapolated value,[d] 9.22 ± 0.01.  [l] Evaluation of primary data by method of J. C. Speakman (J. Chem. Soc., 855 (1940)) with $f_0 = f_1 = f_2 = 1$ to obtain $pK'_A$ values gave $pK'_{A1} = 6.82 ± 0.03$[d] and $pK'_{A2} = 9.10$.  [m] Value given refers to the macroscopic ionization constant.

TABLE II

APPARENT IONIZATION CONSTANTS OF FOUR DERIVATIVES OF GLYCINE AND OF PHENYLALANINE[a]

| Derivative | $pK'_A (NH_3^+)$ Glycine | $pK'_A (NH_3^+)$ Phenylalanine | $\delta$ |
|---|---|---|---|
| Acid | 9.72 | 9.15 | −0.57 |
| Amide | 7.93 | 7.30 | − .63 |
| Hydrazide | 7.69 | 7.12 | − .57 |
| Methyl ester | 7.66 | 7.06 | − .60 |

[a] In aqueous solutions at 25.0 ± 0.1° and 0.05 $M$ in sodium chloride.

nated tyrosine methyl ester was ca. 7.3. With the downward revision of the $pK'_A$ value of the $\alpha$-ammonium group of phenylalanine methyl ester the question arises as to the necessity of revising the earlier estimate of the $pK'_A$ value of the analogous tyrosine derivative, bearing in mind that the principal concern with respect to the magnitude of this constant arose from an inquiry as to the relative abundance of the various species that were of importance in systems whose $pH$ varied from ca. 6.0 to 7.5.[7]  Since these latter conditions limit our interest to the effect of the un-ionized hydroxyl group upon the ionization constant of the $\alpha$-ammonium group we may compare the value of 9.28, i.e., the microscopic ionization constant of the $\alpha$-ammonium group in the dipolar ion containing a carboxylate group and an un-ionized phenolic hydroxyl group,[13] with that of the analogous glycine derivative, i.e., 9.72,[8] to arrive at a difference of 0.44 of a $pK$ unit.[26]  This latter value

(26) The lesser magnitude of this value relative to that obtaining for the glycine–phenylalanine pair, i.e., 0.59 ± 0.04 implies that replace-

can be used to extrapolate the $pK'_A$ value of the $\alpha$-ammonium group in monoprotonated glycine methyl ester, 7.66, to obtain a value of 7.22 for the tyrosine derivative of interest.  The essential agreement of this latter value with that assumed previously[7] does not require significant modification of the argument presented earlier[7] relative to the interpretation of the $pH$–activity relationship observed with the system $\alpha$-chymotrypsin–L-tyrosine ethyl ester.

In the preceding discussion our concern has been with the dependence of the $pK_A$ value of the $\alpha$-ammonium group upon the nature of the $\alpha$-amino acid side chain.  When attention is directed to the dependence of the above constant upon the nature of the carboxyl function it will be seen from the data summarized in Table I that while there is a substantial increase in the acidity of the $\alpha$-ammonium group when $-CO_2^-$ is replaced by $-CONH_2$, i.e., 1.93 $pK_A$ units, the replacement of the latter function by any one of five other functional derivatives of the carboxyl group results in the extreme case in a further increase of only 0.44 of a $pK_A$ unit.  The order of effectiveness of the various carboxyl functions in increasing the acidity of the $\alpha$-ammonium group, i.e., $-CONOH>$ $-CO_2CH_3 \geq -CONHNH_2 \doteq -C(NOH)NH_2 > -C-SNH_2 > -CONH_2 >> -CO_2^-$, is not the one expected if the only factor were the electron deficiency arising from the polarization of the carboxyl carbon

ment of $p$-hydrogen by a phenolic hydroxyl group causes a decrease in the acid strength of the $\alpha$-ammonium group, a result which would be expected.[23]

atom. However, it must be noted that with both the hydroxamide and amidoxime the $pK_A$ values refer to macroscopic ionization constants and with the hydroxamide, hydrazide and amidoxime the possibility of intramolecular hydrogen bonding, leading to an increase in the acidity of the $\alpha$-ammonium group, cannot be excluded. Therefore, if consideration is limited to those derivatives where the above factors cannot intrude, the order observed, with respect to effectiveness in increasing the acidity of the $\alpha$-ammonium group, i.e., $-CO_2CH_3 > -CSNH_2 > -CONH_2 >> -CO_2^-$, is that expected on the basis of an inductive effect arising from a decreasing electron deficiency at the carboxyl carbon atom.

Since there is relatively little information available with respect to the infrared spectra of carboxyl derivatives of the $\alpha$-amino acids the spectra of the seven compounds listed in Table I were determined with a sodium chloride prism for the solid in solid potassium bromide. The results are summarized in the Experimental section which follows. As an unsubstituted benzyl group was present in all of the compounds examined it is possible that the maxima observed at 3040 $\pm$ 20 (6/7),[27] 2937 $\pm$ 30 (6/7), 1615 $\pm$ 12 (7/7), 1500 $\pm$ 4 (7/7), 1454 $\pm$ 7 (7/7), 1155 $\pm$ 20 (5/7), 1074 $\pm$ 5 (7/7), 1024 $\pm$ 9 (7/7), 745 $\pm$ 10 (7/7) and 699 $\pm$ 2 (7/7) cm.$^{-1}$ are a consequence of the presence of this group.[28] Furthermore, the maxima at 1283 $\pm$ 11 (6/7) may be associated with the fact that all of the compounds were $\alpha$-amino acid derivatives.[28] Finally, it should be noted that the spectra observed for DL-phenylalanine are in substantial agreement with those reported by Wright[29] and thus can serve as a point of reference for the other spectra whose interpretation at the present time is premature because of the absence of information with respect to the spectral behavior of analogous compounds lacking the $\alpha$-amino group and/or the aromatic side chain.

### Experimental[30,31]

DL-**Phenylalanine.**—A preparation of synthetic DL-phenylalanine (Dow) was recrystallized twice from water and dried *in vacuo* over phosphorus pentoxide.

DL-**Phenylalanine Methyl Ester.**—Esterification of DL-phenylalanine with methanol and thionyl chloride[32] gave the methyl ester hydrochloride, m.p. 158–159°, after recrystallization from a 3:10 mixture of methanol and ethyl ether; lit.[33] m.p. 158°. The hydrochloride *per se* was used in the determination of the $pK'_A$ value. However, an ethereal solution of the base was prepared, by reaction of an ethereal suspension of the hydrochloride with aqueous sodium bicarbonate, and after the solution had been dried over magnesium sulfate a portion was mixed with potassium bromide and the mixture dried prior to its use for the determination of the infrared spectra.

DL-**Phenylalaninamide.**—Ammonolysis of an ethereal solution of 3.6 g. of DL-phenylalanine methyl ester, prepared from the hydrochloride by reaction with aqueous sodium bicarbonate, gave 2.3 g. of the amide, m.p. 138–

(27) Fraction of compounds examined possessing the specified absorption.

(28) L. J. Bellamy, "Infrared-red Spectra of Complex Molecules," Methuen and Co., London, 1954.

(29) N. Wright, *J. Biol. Chem.*, **127**, 137 (1939).

(30) All melting points are corrected.

(31) Microanalyses by Dr. A. Elek.

(32) M. Brenner and W. Huber, *Helv. Chim. Acta*, **36**, 1109 (1953).

(33) "Beilstein," 4th edition, Vol. 14, System 1905, p. 499.

140°, after recrystallization from chloroform; lit.[34] m.p. 138–139°.

DL-**Phenylalaninthioamide**, m.p. 135–136.3°, was prepared by Peterson and Niemann.[16]

DL-**Phenylalaninhydrazide.**—A mixture of 1.8 g. of DL-phenylalanine methyl ester and 1 g. of anhydrous hydrazine in 25 ml. of absolute ethanol was heated under refluxing conditions to give 1.1 g. of the hydrazide, m.p. 87.5–89.0°. Recrystallization of this product from a mixture of absolute ethanol and hexane gave the hydrazide, m.p. 88.5–90.0°.

*Anal.* Calcd. for $C_9H_{13}ON_3$ (179): C, 60.3; H, 7.3; N, 23.5. Found: C, 60.5; H, 7.4; N, 23.4.

DL-**Phenylalaninamidoxime** ($\alpha$-**amino-$\beta$-phenylpropioamidoxime**), m.p. 117.5–118.5°, was prepared by Peterson and Niemann.[16]

DL-**Phenylalaninhydroxamide.**—DL-Phenylalanine methyl ester hydrochloride, 10.8 g., was converted into the hydroxamide as directed by Cunningham, *et al.*,[35] the crude product recrystallized from water and dried *in vacuo* over phosphorus pentoxide, at 56° to give *ca.* 4 g. of the hydroxamide, m.p. 180–181.2°, lit.[35] m.p. 180° dec.

**Potentiometric Determination of Apparent Ionization Constants.**—All measurements were made with a difunctional recording titrator designed and built by M. D. Cannon, International Instruments Co., Canyon, Calif. The instrument is a modification of that described by Neilands and Cannon[17] and contains the elements essential for either constant or variable $pH$ titrations. In the titrations described herein the instrument was calibrated and set for variable $pH$ titration. When used in this manner the synchronous motor, operated at 6 r.p.m., delivered the titrating fluid at the rate of 30 $\times$ 10$^{-3}$ ml./min. The base employed in these titrations was of high enough concentration as to cause a negligible change in the volume of the solution during the titration. With 0.8534 $N$ NaOH the delivery rate corresponds to 25.63 microequivalents per min. The base was delivered to the solution through a Dewitt and Herz Inc. stainless steel hypodermic needle with the tip bent up to limit diffusion of the base into the solution. The cell was of 25-ml. capacity and was enclosed in a thermostated water jacket in order to maintain the temperature at 25.0 $\pm$ 0.1°. The solution was vigorously stirred and a $CO_2$-free atmosphere was maintained by "sweeping" the solution with nitrogen. The $pH$ of the solution was measured using a Beckman no. 4990–29 glass electrode and no. 4970–29 calomel reference electrode in conjunction with a Leeds and Northrup model 7664–41 $pH$ meter. The meter output was coupled with a Leeds and Northrup Speedomax type G recorder to obtain a titration curve directly. In all cases the electrodes were standardized with buffer solution at $pH$ 4, 7 and 10 before and after the titration and correction made for the non-linearity of this calibration. In all cases standard stock solutions were prepared in $CO_2$-free water as was the base and acid. Ten ml. aliquots of stock solutions of the amino acid derivatives containing approximately 75 microequivalents per 10 ml. were pipetted into the reaction cell, and 10 ml. of the various salt solutions (0.4, 0.2, and 0.1 $M$) were added. One or two ml. of 0.0498 $N$ hydrochloric acid was pipetted into the solution and the now acid solution titrated as indicated above. Further experimental details are given in Table I.

**Infrared Spectra.**—The infrared spectra of the seven compounds listed in Table I were determined with a Perkin–Elmer model 21 spectrophotometer, equipped with a sodium chloride prism, for the solid in solid potassium bromide and were as follows: hydroxamide, 3185(m), 3040(s), 2882(s), 2604(m), 2137(w), 1647(m), 1616(s), 1550(m), 1497(w), 1460(m), 1379(s), 1335(w), 1290(s), 1199(w), 1167(m), 1071(w), 1033(w), 1001(w), 963(w), 912(m), 887(m), 860-(w), 771(w), 755(m), 733(w), 697(s), 682(w); methyl ester, 3390(w), 3021(w), 2950(m), 1742(s), 1608(w), 1590(w), 1499(m), 1456(m), 1439(s), 1374(w), 1272(m), 1196(s), 1168(s), 1099(w), 1073(w), 1028(w), 1005(m), 870(w), 835(m), 812(w), 744(s), 701(s); hydrazide, 3344(m), 3289-(m), 2994(w), 2915(w), 1678(s), 1656(s), 1618(s), 1515(s), 1497(m), 1458(w), 1443(w), 1397(w), 1282(w), 1071(w), 1029(w), 977(w), 943(w), 925(m), 901(m), 882(w), 753(m), 700(s); amidoxime, 3425(s), 3311(s), 3165(s), 3106(m),

(34) Reference 33, 2nd supplement, p. 606.

(35) K. G. Cunningham, G. T. Newbold, F. S. Spring and J. Stark, *J. Chem. Soc.*, 2091 (1949).

3049(m), 2933(m), 2770(m), 1667(s), 1603(s), 1497(m), 1456(w), 1403(w), 1175(w), 1073(w), 1028(w), 977(w). 912(s), 850(w), 827(w), 748(m), 698(s); thioamide, 3311(s). 3279(s), 3021(m), 2907(m), 2817(m), 1692(w), 1681(w), 1664-(w), 1647(m), 1634(m), 1603(m), 1582(m), 1536(w), 1497-(m), 1464(s), 1458(s), 1362(w), 1311(w), 1272(w), 1205-(w), 1068(w), 1032(w), 1015(w), 987(w), 934(m), 885(m), 754(s), 735(m), 698(s), 676(w), 672(w): amide, 3311(s). 3058(s), 2950(m), 2817(w), 1675(s), 1613(s), 1595(s), 1499-(m), 1451(m), 1416(s), 1361(w), 1335(m), 1302(w), 1284-

(w), 1214(w), 1134(m), 1117(m), 1079(m), 1029(w), 1000-(s), 953(s), 928(w), 903(m), 873(w), 849(w), 779(s), 766-(w), 734(s), 700(s); acid, 3448(w), 3040(m), 2967(m), 2710-(m), 2525(m), 2151(m), 1626(s), 1587(s), 1513(s), 1504(s), 1447(m), 1414(s), 1340(m), 1309(s), 1294(m), 1208(w), 1155(w), 1129(w), 1071(w), 1032(w), 984(w), 912(w), 852-(m), 775(w), 745(m), 697(s), 677(w), with all values in cm.$^{-1}$ and with the intensity indicated as strong (s), medium (m) and weak (w).

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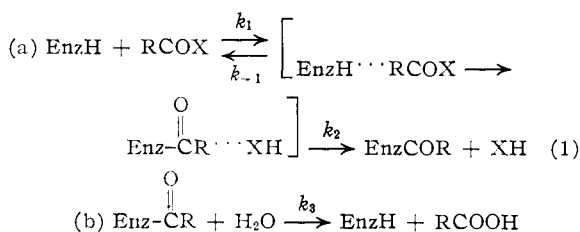
# Imidazole Catalysis. V. The Intramolecular Participation of the Imidazolyl Group in the Hydrolysis of Some Esters and the Amide of γ-(4-Imidazolyl)-butyric Acid and 4-(2'-Acetoxyethyl)-imidazole[2]

BY THOMAS C. BRUICE[3] AND JULIAN M. STURTEVANT

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The synthesis of a number of imidazoles including γ-(4-imidazolyl)-butyric acid (V) and four of its phenyl esters as well as the methyl ester and amide are recorded. Also, a new method for the easy preparation of N-acyl imidazoles is noted. The phenyl esters of V solvolyze rapidly in water due to the very effective anchimeric assistance of the neutral imidazolyl group. The fact that the p-nitrophenyl ester of V exhibits a rate of solvolysis almost identical to that of the α-chymotrypsin-p-nitrophenyl acetate complex is discussed in the light of the involvement of an imidazolyl group in both processes. The change in mechanism in going from inter- to intramolecular catalysis of substituted phenyl acetates is discussed in terms of the nucleophilic attack becoming concerted with the dissociation of the imidazolium species so that the rate-determining step becomes the collapse of the tetrahedral intermediate in the intramolecular reactions whereas in the bimolecular reactions the tetrahedral intermediate is at a very low and steady state concentration. Unlike the methyl ester of V, 4-(2'-acetoxy-ethyl)-imidazole undergoes hydrolysis with imidazole participation. This is the first reported instance of the nucleophilic catalysis of the hydrolysis of an aliphatic ester by an imidazole. In the hydrolysis of the amide of V the protonated imidazolyl group participates. The similarity between the effectiveness of the imidazole and carboxyl anion and imidazolium and carboxyl groups as anchimeric participants in ester and amide hydrolysis is pointed out.

Considerable evidence has been accumulated in recent years to indicate that esters and amides are catalytically hydrolyzed by esteratic enzymes through a double displacement reaction involving an acylated enzyme intermediate. The formation

$$\text{(a)} \quad \text{EnzH} + \text{RCOX} \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} \left[ \text{EnzH}\cdots\text{RCOX} \longrightarrow \right.$$

$$\left. \underset{\text{Enz-CR}}{\overset{\text{O}}{\|}}\cdots\text{XH} \right] \overset{k_2}{\longrightarrow} \text{EnzCOR} + \text{XH} \quad (1)$$

$$\text{(b)} \quad \underset{\text{Enz-CR}}{\overset{\text{O}}{\|}} + \text{H}_2\text{O} \overset{k_3}{\longrightarrow} \text{EnzH} + \text{RCOOH}$$

of acyl-enzyme in 1a takes place after formation of an enzyme–substrate complex, and undoubtedly involves the participation of specific amino-acid side chains (intracomplex participation) in the displacement of X; in at least one case[4] the formation of acyl-enzyme is kinetically first order in the enzyme–substrate complex. It follows, therefore, that appropriate models for esteratic enzymes should be sought among hydrolytic reactions which proceed via first-order processes with assistance of an intracomplex or intramolecular nature. Intracomplex participation, in the catalysis of the hydrolysis of amides and esters, has been

realized through incorporation of the nucleophilic or electrophilic participants into polymers to which the substrate becomes bound.[5,6] Due to the ready availability of suitably substituted esters and amides, the carboxyl and carboxylate groups have received particular attention as intramolecular participants in ester and amide hydrolysis.[7–17]

In the case of numerous esteratic enzymes, there is much evidence to indicate that a non-protonated imidazolyl group of a histidine residue[18] and an aliphatic hydroxyl group of a serine residue[19]

(1) Contribution No. 1523.

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(3) Inquiries to this author should be addressed to the Department of Physiological Chemistry, The Johns Hopkins School of Medicine, Baltimore 5, Md.

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