The Hydrolysis of Peptide Bonds by Intramolecular Participation of Benzimidazolium Ions

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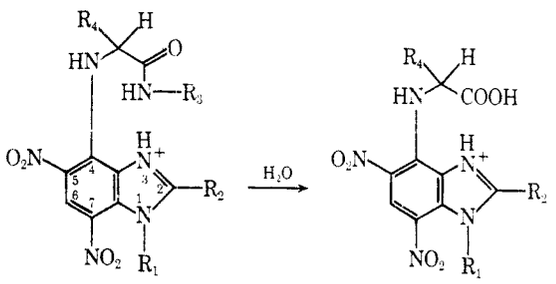
A series of 5,7-dinitrobenzimidazolyl-4 peptides have been prepared and the rates of hydrolysis of the peptide bond determined as a function of acidity and temperature. It is demonstrated that the rate of hydrolysis is dependent on the degree of protonation of a benzimidazole nitrogen atom. The effectiveness of the intramolecular participation varies with the nature of the peptide side chain and with the degree of alkylation of the imidazole ring. No correlation is observed between pK_a and specific rate constant for the various substrates. Heats of ionization and activation parameters are reported for two cases. The compound, 1,2-dimethyl-5,7-dinitrobenzimidazolyl-4-alanylglycine, was found to hydrolyze 65,000 times as rapidly as 2,4-dinitrophenylalanylglycine.

In the preceding paper,[1] we reported on the synthesis of several 5,7-dinitro-4-fluorobenzimidazoles and on the kinetics of displacement of the fluorine atom by simple dipeptides. Using these reagents, we have now prepared a variety of dipeptide derivatives[2] and have studied the rates of peptide bond cleavage as a function of acidity and temperature. This work was undertaken for diverse reasons: we hoped to utilize the bifunctional



1, $R_1 = R_2 = H$
2, $R_1 = H; R_2 = CH_3$
3, $R_1 = R_2 = CH_3$

+ R_3NH_2

nature of the reagents as a means of effecting facile, selective, and quantitative removal of N-terminal residues from polypeptides, providing an alternative to currently available methods;[3] secondly, we planned to use this study as an entry into the general problem of intramolecularly facilitated hydrolysis of peptide bonds.

In contrast to the extensive literature on the intramolecularly catalyzed hydrolysis of esters,[4] significantly less work has been done on the analogous hydrolysis of amides. Of the accessory functional groups which have been investigated in the latter case, the principal efforts have involved the hydroxyl[5] and carboxyl[6] groups. Nitrogen participation has been studied in the form of imidazole,[7] pyridine,[8] pyridine N-oxide,[9] aromatic amine,[10] and amide.[11] Sulfur participation has been demonstrated for thioamides,[12a] thioureas,[12b] and dithiocarbamates.[12c]

There is sufficient evidence for a lack of parallelism in the chemistries and hydrolysis mechanisms of esters and amides (particularly in acidic media) to warrant independent investigation of the latter. For example, differences in resonance stabilization, basicity, and conformation are well known. The fact that esters show ^{18}O exchange in acidic media,[13] while amides generally do not,[14] indicates mechanistic differences;

(1) K. L. Kirk and L. A. Cohen, *J. Org. Chem.*, **34**, 395 (1968).

(2) The alternative order of numbering the benzimidazole ring system has been omitted for the sake of simplicity. In tables or figures, standard abbreviations for amino acids have been used: glycine = gly or G; alanine = ala or A; valine = val or V.

(3) J. I. Harris and V. M. Ingram in "Analytical Methods of Protein Chemistry," P. Alexander and R. J. Block, Ed., Pergamon Press, New York, N. Y., 1960, Chapter 12; J. L. Bailey, "Techniques in Protein Chemistry," Elsevier Publishing Co., New York, N. Y., 1962, Chapter 6.

(4) T. C. Bruice and S. J. Benkovic, "Bioorganic Mechanisms," Vol. I, W. A. Benjamin, Inc., New York, N. Y., 1966, Chapter 1.

(5) (a) M. L. Wolfrom, R. B. Bennett, and J. D. Crum, *J. Amer. Chem. Soc.*, **80**, 944 (1958); (b) H. Zahn and L. Zürn, *Ann. Chem.*, **613**, 76 (1958); (c) L. Zürn, *ibid.*, **631**, 56 (1960); (d) T. C. Bruice and F. H. Marquardt, *J. Amer. Chem. Soc.*, **84**, 365 (1966); (e) T. C. Bruice and D. W Tanner, *J. Org. Chem.*, **30**, 1668 (1965); (f) R. B. Martin, R. Hedrick, and A. Parcell, *ibid.*, **29**, 158 (1964); (g) L. Farber and L. A. Cohen, *Biochemistry*, **5**, 1027 (1966); (h) B. A. Cunningham and G. L. Schmir, *J. Amer. Chem. Soc.*, **89**, 917 (1967).

(6) (a) S. J. Leach and H. Lindley, *Trans. Faraday Soc.*, **49**, 915, 921 (1953); (b) M. L. Bender, Y. L. Chow, and F. Chloupek, *J. Amer. Chem. Soc.*, **80**, 5380 (1958); (c) B. Zerner and M. L. Bender, *ibid.*, **83**, 2267 (1961); (d) H. Morawetz and J. Shafer, *ibid.*, **84**, 3783 (1962); (e) E. H. Westhead, Jr., and H. Morawetz, *ibid.*, **80**, 237 (1958); (f) B. Vigneron-Voortman, P. Crooy, and A. Bruylants, *Bull. Soc. Chim. Belges*, **73**, 241 (1964); (g) B. Witkop, *Advan. Protein Chem.*, **16**, 229 (1961).

(7) T. C. Bruice and J. M. Sturtevant, *J. Amer. Chem. Soc.*, **81**, 2860 (1959).

(8) (a) A. Signor and E. Bordignon, *J. Org. Chem.*, **30**, 3447 (1965); (b) A. Signor, E. Bordignon, and G. Vidali, *ibid.*, **32**, 1135 (1967); (c) A. Signor, L. Biondi, and E. Bordignon, *ibid.*, **31**, 1403 (1966).

(9) (a) V. Tortorella and G. Tarzia, *Gazz. Chim. Ital.*, **97**, 1479 (1967); (b) V. Tortorella and G. Bettoni, *ibid.*, **97**, 1487 (1967).

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(11) (a) M. Vigneron, P. Crooy, F. Kezdy, and A. Bruylants, *Bull. Soc. Chim. Belges*, **69**, 616 (1960); (b) P. Crooy and A. Bruylants, *ibid.*, **73**, 44 (1964); (c) T. Cohen and J. Lipowitz, *J. Amer. Chem. Soc.*, **86**, 5611 (1964); (d) J. A. Shafer and H. Morawetz, *J. Org. Chem.*, **28**, 1899 (1963); (e) G. R. Stark and D. G. Smyth, *J. Biol. Chem.*, **238**, 214 (1963).

(12) (a) G. C. Barrett, *Chem. Commun.*, 487 (1967); G. W. Kenner and H. G. Khorana, *J. Chem. Soc.*, 2076 (1952); (b) P. Edman, *Acta Chem. Scand.*, **10**, 761 (1956); (c) A. L. Levy, *J. Chem. Soc.*, 404 (1950); J. Leonis, *Bull. Soc. Chim. Belges*, **61**, 524 (1952); D. T. Elmore and P. A. Toseland, *J. Chem. Soc.*, 4533 (1954).

(13) M. L. Bender, *J. Amer. Chem. Soc.*, **73**, 1626 (1951).

(14) (a) M. L. Bender and R. D. Ginger, *ibid.*, **77**, 348 (1955); (b) C. A. Bunton, C. O'Connor, and T. A. Turney, *Chem. Ind.* (London), 1835 (1967).

TABLE I

5,7-DINITROBENZIMIDAZOLYL-4 AMINO ACIDS AND DIPEPTIDES

Compound	Mp, °C[a]	Formula	Calcd, % C	Calcd, % H	Calcd, % N	Found, % C	Found, % H	Found, % N	Yield, %	R_f[b]
1-DL-ala	208–213	$C_{10}H_9N_5O_6$	40.68	3.07	23.73	40.68	3.27	23.98	92	0.79
2-gly	230–238	$C_{10}H_9N_5O_6$	40.68	3.07	23.73	40.82	3.06	23.94	90	0.53
2-DL-ala	222–240	$C_{11}H_{11}N_5O_6$	42.87	3.59	22.65	43.00	3.89	22.59	43	0.83
2-L-val	195–198	$C_{13}H_{15}N_5O_6$	46.29	4.48	20.77	46.33	4.29	20.54	33	0.90
3-gly[c]	215–222	$C_{11}H_{11}N_5O_6$		*m/e* 309			*m/e* 309		97	0.70
3-DL-ala[c]	230–245	$C_{12}H_{13}N_5O_6$		*m/e* 323			*m/e* 323		50	0.84
1-L-alagly	225–240	$C_{12}H_{12}N_6O_7$	40.91	3.43	23.86	40.87	3.25	23.92	73	0.20
2-L-alagly	220–230	$C_{13}H_{14}N_6O_7$	42.62	3.85	22.95	42.82	3.77	23.04	82	0.28
2-gly-DL-ala	220–229	$C_{13}H_{14}N_6O_7$	42.62	3.85	22.95	42.60	4.04	22.66	77	0.27
2-glygly	242–245	$C_{12}H_{12}N_6O_7$	40.91	3.43	23.86	41.20	3.57	23.67	90	0.11
2-L-ala-L-ala	241–245	$C_{14}H_{16}N_6O_7$	44.21	4.24	22.10	44.27	4.52	21.95	76	0.47
2-L-valgly	189–192	$C_{15}H_{18}N_6O_7$	45.68	4.60	21.31	45.39	4.74	21.07	50	0.50
2-L-ala-L-val	240–244	$C_{16}H_{20}N_6O_7$	47.06	4.94	20.58	46.96	4.77	20.49	87	0.76
3-L-alagly	195–200	$C_{14}H_{16}N_6O_7$	44.21	4.24	22.10	44.39	4.54	21.68	79	0.39
3-gly-DL-ala[c]	220–227	$C_{14}H_{16}N_6O_7$		*m/e* 380			*m/e* 380		55	0.38

[a] All compounds melt with decomposition. [b] Tlc on silica gel GF, developed with chloroform–*t*-amyl alcohol–acetic acid (70:30:5). [c] Composition ascertained by parent peak in mass spectrum because of difficulties in crystallization.

furthermore, a dependence of mechanism on the nature of the leaving group has been demonstrated for certain esters.[15] It may be expected that intramolecular hydrolysis will be even more sensitive to structural variations. Finally, the extent to which the mass of data and speculation on the enzymatic hydrolysis of esters[4] is relavant to amide substrates remains uncertain.

Experimental Section[16]

Materials.—Dipeptides (Mann Research Laboratories) were coupled with dinitrofluorobenzimidazoles following the preparative procedure previously reported.[1] Analytical and physical data for these compounds are recorded in Table I. For reference purposes, the corresponding amino acid derivatives were prepared in a similar manner. In each case, recrystallization was effected from aqueous ethanol and homogeneity checked by thin layer chromatography (tlc) (Table I).

pK_a' Measurements.—Standardized hydrochloric acid (2.00 *N*) was diluted as necessary with 2.00 *M* potassium chloride. The solutions were then diluted with ethanol (9:1, v/v) containing *o*-nitroaniline and H_0' values[17] determined spectrophotometrically at 25°.[18] The pK_{BH+} value for the indicator was taken as −0.29, neglecting variations due to the added salt or ethanol. The results are given in Table II; values of H_0 in dilute hydrochloric acid, obtained by interpolation of the data of Paul and Long,[18] are included for comparison.

Using similar procedures and the values of H_0' obtained above, pK_a' (25°) values were determined for peptide derivatives of the several dinitrobenzimidazoles, as recorded in Table III. The uv spectra of the benzimidazole chromophores are quite characteristic, both in the neutral and acidic forms; an example, **2**-alanine, is shown in Figure 1. Primarily, the absorbance at 310 mμ, at various values of H_0', was used for pK_a' calculations. Within the range 25–50°, the desired temperature was maintained by circulation of thermostated water through the cell holder.[19]

TABLE II

ACIDITIES OF HYDROCHLORIC ACID SOLUTIONS (μ = 2.00) CONTAINING 10% ETHANOL (BY VOLUME) AT 25°

Acid concentration, mol/l.	H_0'[a]	H_0[b]
0.18	0.54	0.75
0.27	0.40	0.52
0.36	0.27	0.40
0.45	0.14	0.27
0.68	−0.03	0.03
0.90	−0.17	−0.13
1.35	−0.38	−0.39
1.80	−0.51	−0.60

[a] Values determined in this investigation. [b] Values for aqueous hydrochloric acid, based on ref 18.

TABLE III

RATES OF HYDROLYSIS OF 5,7-DINITROBENZIMIDAZOLYL-4 DIPEPTIDES[a]

Compound	pK_a' (25°)	$k_{obsd} \times 10^2$, min^{-1}	$k_a \times 10^2$,[b] min^{-1}	Relative rates
DNP-alagly			0.0021[c]	1
1-alagly	−0.69	5.68	31.9	15200
2-alagly	0.25	18.78	28.7	13700
2-alala	0.15	7.16	11.9	5700
2-glygly	0.62	2.27	2.8	1300
2-glyala	0.59	1.52	1.9	900
2-alaval	0.03	1.84	3.5	1700
2-valgly	0.02	0.65	1.2	600
3-alagly	−0.29	48.10	136.6	65000
3-glyala	0.18	4.87	7.9	3800
2-gly	0.39			
2-ala	0.20			
2-val	−0.10			

[a] At 50°, H_0' = −0.03. [b] Calculated using pK_a' (25°) and eq 2. [c] Based on data of Signor and Bordignon;[8a] rate of hydrolysis measured under (pseudo) first-order conditions, at 60°. For conversion to 50°, E_a was taken as 20 kcal/mol.

In the cases of **2**- and **3**-alanylglycine, extrapolation to zero time was made prior to calculation, since some hydrolysis occurs at 25°.

Rate Measurements.—Solutions of hydrochloric acid (1.80 ml), at constant ionic strength (2.00 *M*), were placed in glass-stoppered vessels and brought to the desired temperature in a thermostated water bath. Solutions of the substrate in ethanol

(15) J. W. Thanassi and T. C. Bruice, *J. Amer. Chem. Soc.*, **88**, 747 (1966).

(16) Ultraviolet (uv) spectra were measured using a Cary recording spectrophotometer, Model 14. High resolution mass spectra were measured on an Hitachi double-focusing spectrometer, Model RMU-6E. Microanalyses were performed by Dr. W. C. Alford and his associates of this institute.

(17) H_0 and pK_a' refer specifically to media of fixed ionic strength (2.00 *M*) and containing 10% ethanol, as used throughout the present study.

(18) M. A. Paul and F. A. Long, *Chem. Rev.*, **57**, 1 (1957).

(19) No correction was made for the variation of H_0', with temperature. Within the temperature range used, the correction is estimated not to exceed 0.05 H_0 units [A. I. Gelbshtein, G. G. Shcheglova, and M. I. Temkin, *Dokl. Akad. Nauk SSSR*, **107**, 108 (1956)].

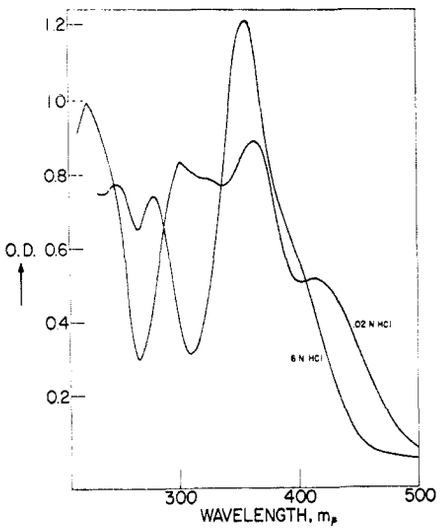


Figure 1.—The uv spectra of 2-alanine in neutral and acidic media.

(0.20 ml, *ca.* 1.5×10^{-2} *M*) were added and 0.10-ml aliquots were withdrawn periodically. The liberated amino acid was assayed by the ninhydrin method,[20] aqueous base being added where necessary to neutralize excess hydrochloric acid. Infinity values were obtained by continuing the hydrolysis runs until the ninhydrin assay gave constant values. Upon completion of a run, the dinitrobenzimidazolylamino acid was the only colored component detectable by tlc. Control experiments showed the dinitrobenzimidazolylamino acids to be completely stable under the conditions of hydrolysis. Dinitrobenzimidazolyl peptides are stable to hydrolysis under alkaline conditions; no peptide bond cleavage was detectable at 50° and pH 10.

Results and Discussion

pK_a' Measurements.—The basicity of the benzimidazole ring system is markedly dependent on the nature of the attached peptide (Table III), as well as on the degree of alkylation of the heterocyclic ring. From the limited cases examined, it would appear that the N-terminal amino acid is the principal determinant of basicity, the pK_a' (25°) values for derivatives of glycyl peptides being 0.4–0.5 units higher than those for alanyl peptides; similar variations are observed in the pK_a' values of the amino acid derivatives. The values bear no relationship, in their order, to the pK_a values of the simple dipeptides and cannot be explained on the basis of inductive effects. More likely, the variation may stem from a combination of electrostatic interactions and the conformational orientation of the peptide side chain, the latter possibly being influenced by the 5-nitro group. In any case, it is clear that, since rates of hydrolysis depend on the degree of protonation of the imidazole nitrogen (see below), variations in basicity must be considered in any comparisons made.

The introduction of a 2-methyl substituent increases benzimidazole basicity by one pK unit (2-alanylglycine *vs.* 1-alanylglycine). Such an enhancement is slightly greater than that observed with simpler benzimidazoles (*ca.* 0.8 unit).[21] The introduction of a 1-methyl substituent normally has little effect on the basicities of benzimidazoles or 2-methylbenzimidazoles. In the present series, the 1,2-dimethyl derivatives (**3**-alanylglycine, **3**-glycylalanine) are less basic than their 2-methyl counterparts by 0.4–0.5 pK units, a phenomenon which may be due to steric interaction between the 7-nitro and 1-methyl groups[1] and the resulting distortion of the imidazole ring.

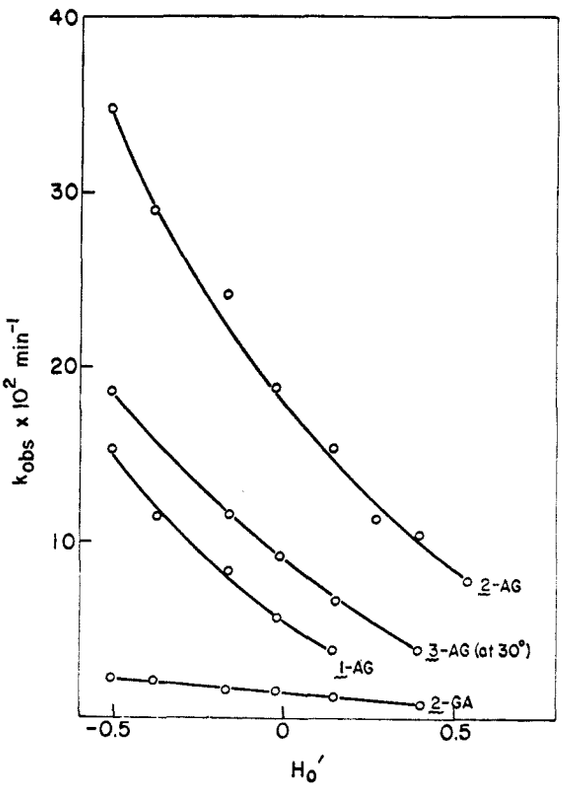


Figure 2.—Dependence of hydrolysis rates on H_0' at 50°.

Rates of Hydrolysis.—The rates of hydrolysis of the various benzimidazolyl dipeptides were followed to 60–90% completion, the first-order rate law being obeyed over the entire range. Observed (pseudo) first-order rate constants are recorded in Table III.[22] The variation of rate with H_0', as shown in Figure 2 for representative compounds, suggests participation by the benzimidazolium species. Since the benzimidazole group can exist in the protonated (ImH^+), neutral (Im), and anionic (Im^-) species (except for series **3**), the total rate may be expressed as a sum of terms (eq 1). Since no peptide bond cleavage was

$$k_{obs}[Im_{tot}] = k_a[ImH^+] + k_n[Im] + k_b[Im^-] \quad (1)$$

observed in alkaline media, $k_b = 0$; the absence of any deviation from linearity in Figure 3 (see below) indicates the acid-independent rate, k_n, to be 0 or negligible. Thus

$$k_{obsd} = k_a[ImH^+/Im_{tot}] = k_a \frac{h_0'}{h_0' + K_a'} \quad (2)$$

(20) D. H. Spackman, W. H. Stein, and S. Moore, *Anal. Chem.*, **30**, 1190 (1958).

(21) K. Hofmann, "Imidazole and Its Derivatives," Interscience Publishers, New York, N. Y., 1953, p 251.

(22) All rate constants and other slope values were calculated by the method of least squares.

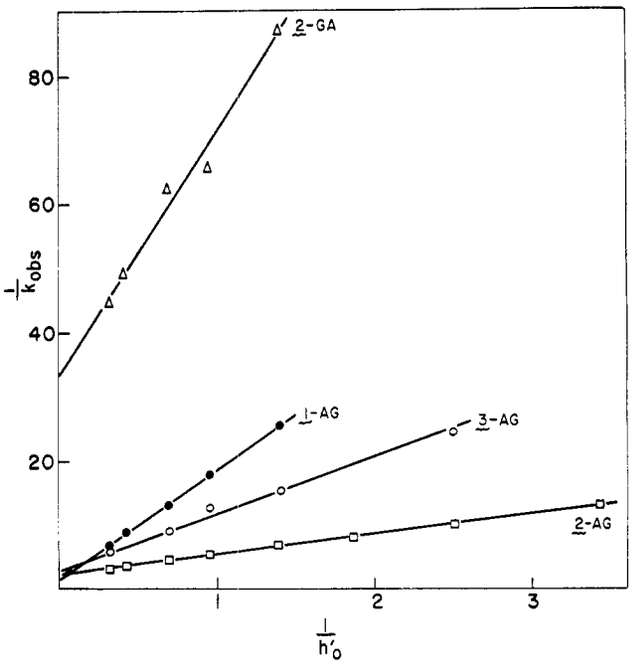


Figure 3.—Plot of $1/k_{obsd}$ *vs.* $1/h_0'$ as a test for kinetic dependence on protonated benzimidazole.

and

$$1/k_{obsd} = 1/k_a + K_a'/k_a \times 1/h_0' \quad (3)$$

where k_a = the specific rate constant for the acid-catalyzed reaction and K_a' = the apparent dissociation constant for the equilibrium 4.

$$-NH^+ \overset{K_a'}{\rightleftharpoons} -N + H^+ \quad (4)$$

For each compound studied, the linearity of a plot of $1/k_{obsd}$ *vs.* $1/h_0'$ (Figure 3) demonstrated a kinetic dependence on the concentration of protonated benzimidazole.[23] No attempt was made to derive values of k_a or K_a' from eq 3 and the slopes of the lines in Figure 3, since such values are highly sensitive to slight variations in slope. Approximate values of k_a (Table III) were calculated from k_{obsd} and pK_a' (25°). For two compounds, 2-alagly and 2-glyala, the variation of pK_a' with temperature was determined spectrophotometrically; the results, together with thermodynamic functions, are shown in Table IV. The values are in accord with those obtained for other very weak bases, such as *o*- and *p*-nitroaniline.[24] The error in k_a (50°), introduced by neglecting the difference between pK_a' (25°) and pK_a' (50°), would amount to 10–15% and would not significantly affect the conclusions drawn from these results.

A Brønsted plot of log k_a (50°) *vs.* pK_a' (25°) (Figure 4) fails to show any general dependence of hydrolysis rate on basicity, except for the three 2-alanyl-X compounds, which obey a linear relationship. On the other hand, the specific rates for 3-alanylglycine and 3-glycylalanine show an inverse dependence on benzimidazole basicity. The order of hydrolysis rates for the series of 2 dipeptides bears no relationship to that for hydrolysis of the simple dipeptides,[25] possibly because the intramolecular reaction is governed principally by conformational alignment of the side chain, rather than by steric accessibility to water.

Variation in the benzimidazole portion of the molecule similarly fails to demonstrate any trend. Of the three alanylglycine derivatives studied, 3-alanylglycine hydrolyzes most rapidly, although its pK_a' value falls between those for the corresponding 1- and 2-alanylglycine derivatives. Although 2-methylimidazoles and 2-methylbenzimidazoles are significantly less nucleophilic than their unsubstituted counterparts,[26] presumably for steric reasons, such a factor is not obvious in the present study. It is well known, however, that steric hindrance is far less of a deterrent to intramolecular than to intermolecular reactions.[27] Furthermore, while the benzimidazole anion is a better nucleophile than the neutral species toward *p*-nitrophenyl acetate,[26] it is ineffective toward amide substrates.

Effect of Temperature.—Rates of hydrolysis at a series of temperatures were determined for 2-alanylglycine and 2-glycylalanine. Based on heats of ionization, values of pK_a' at each temperature were calculated and, in turn, values of k_a from k_{obsd} and pK_a' (eq 2).[19]

TABLE IV
APPARENT HEATS OF IONIZATION OF BENZIMIDAZOLYL DIPEPTIDES

Compound	pK_a' (25°)	pK_a' (50°)[a]	$\Delta H'_{ionizn}$, cal/mol	ΔS_{ionizn}, eu
2-alagly	0.25	0.12	2600	7.4
2-glyala	0.59	0.38	3600	9.4

[a] pK_a' was also determined at several intermediate temperatures.

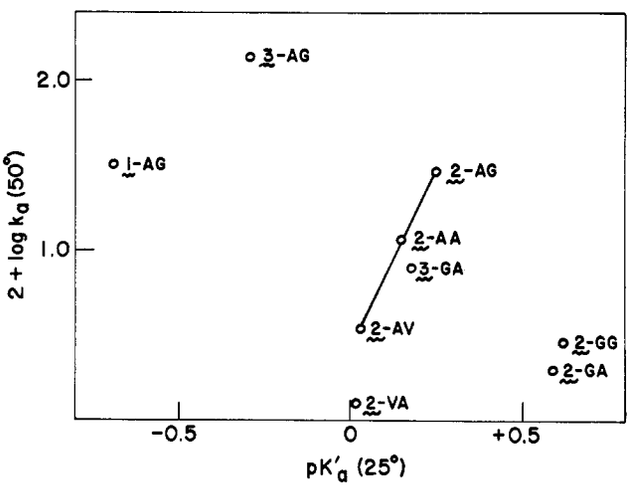


Figure 4.—Variation of specific rate constants (k_a) with basicities of benzimidazoles.

(23) For most of the acid concentrations used, the deviation of h_0 from $a_{H_3O^+}$ is small.

(24) Thus, *o*-nitroaniline (pK_a = −0.26) shows ΔH = 1710 cal/mol and ΔS = 6.9 eu and *p*-nitroaniline (pK_a = 1.00), ΔH = 3130 cal/mol, and ΔS = 5.9 eu [A. I. Biggs, *J. Chem. Soc.*, 2572 (1961)].

(25) R. L. Hill, *Advan. Protein Chem.*, **20**, 37 (1965).

(26) T. C. Bruice and G. L. Schmir, *J. Amer. Chem. Soc.*, **80**, 148 (1958).

(27) (a) E. L. Eliel, "Steric Effects in Organic Chemistry," M. S. Newman, Ed., John Wiley & Sons, Inc., New York, N. Y., 1956, p 119; (b) R. M. Topping and D. E. Tutt, *J. Chem. Soc., B*, 1346 (1967).

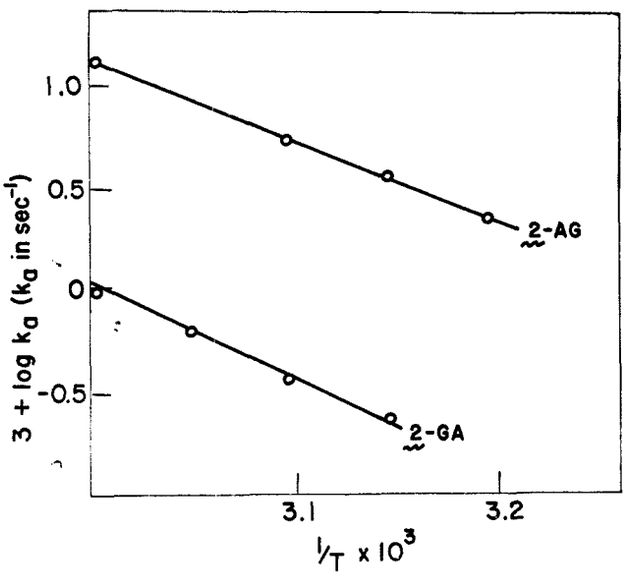


Figure 5.—Effect of temperature on specific rate constants for hydrolysis ($H_0' = -0.03$).

Plots of log k_a *vs.* $1/T$ were linear (Figure 5), and provided the activation parameters summarized in Table V. The small negative entropies of activation are indicative of the intramolecular nature of the rate-determining step in the hydrolytic reaction and, possibly, of the noninvolvement of water in that step.[28]

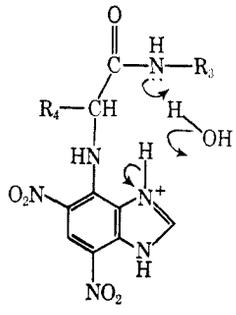
TABLE V

ACTIVATION PARAMETERS FOR BENZIMIDAZOLYL DIPEPTIDES[a]

Compound	E_a, kcal/mol	$\Delta H^{\ddagger}$, kcal/mol	$T\Delta S$, kcal/mol	$\Delta F^{\ddagger}$, kcal/mol
2-alagly	18.7	18.2	−3.3	21.4
2-glyala	21.4	20.7	−4.2	24.9

[a] Calculated for 50°.

Reaction Mechanism.—It would be premature to attempt to select from the various mechanisms which may be considered for the intramolecular facilitation of hydrolysis by the benzimidazolium species. Geometrical limitations do not favor the benzimidazolium ion acting as a general acid (**4**). The formation of cyclic acyl intermediates has neither been demonstrated nor excluded: the fact that 3-alanylglycine and 3-glycylalanine hydrolyze 4–5 times as rapidly as 2-alanylglycine and 2-glycylalanine, respectively, makes consideration of an acylbenzimidazole intermediate difficult; on the other hand, the dimethylbenzimidazole series may operate by a pathway different from that of the less alkylated series. Regardless of the detailed mechanism, the facilitation effect includes the entropy advantage of an intramolecular reaction, an increase in the local concentration of protons, and, possibly, some conformational control in the proton-transfer process. Of the cases examined, that of 3-alanylglycine is most impressive, its k_a being 65,000 times as great as that of 2,4-dinitrophenylalanylglycine.



4

Registry No.—1-DL-ala, 18646-22-5; 1-L-alagly, 18645-99-3; 2-gly, 18646-24-7; 2-DL-ala, 18646-25-8; 2-L-val, 18646-26-9; 2-L-alagly, 18646-00-9; 2-gly-DL-ala, 18646-28-1; 2-glygly, 18646-29-2; 2-L-ala-L-ala, 18646-30-5; 2-L-valgly; 18646-31-6; 2-L-ala-L-val, 18646-32-7; 3-gly, 18646-33-8; 3-DL-ala, 18646-34-9; 3-L-alagly, 18646-01-0; 3-gly-DL-ala, 18646-36-1.

(28) *Cf.* E. Gaetjens and H. Morawetz, *J. Amer. Chem. Soc.*, **82**, 5328 1960), and J. W. Thanassi and T. C. Bruice, *ibid.*, **88**, 747 (1966).