The Effect of Structure on Kinetics of the Acid Hydrolysis of Nitro-2-pyridyl Dipeptides

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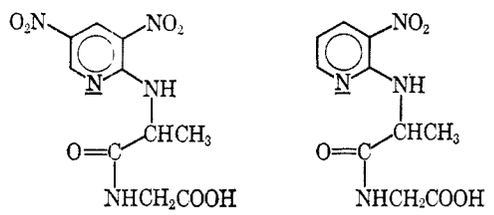
The kinetics of the acid-catalyzed hydrolyses of 3- and 5-nitro-2-pyridylalanylglycine have been determined at several temperatures; the activation parameters of these processes have been calculated. The rates of the catalyzed hydrolyses are found to be H_0 dependent, and it is postulated that the effective catalyst is the protonated pyridine species. The rate constants of hydrolysis of 3-nitro-2-pyridylalanylglycine, despite the rather smaller basicity of the heterocyclic nitrogen, are significantly greater than those of 5-nitro-2-pyridylalanylglycine; this is ascribed to an effect of steric hindrance in the ground state and, more tentatively, to a stabilization of the transition state by interaction of the amino hydrogen with the oxygen of the *o*-nitro group. Furthermore kinetic measurements performed on 3-nitro-2-pyridyl-β-alanylglycine demonstrate that the elongation of the side chain causes a marked decrease in the observed rate constants of hydrolysis.

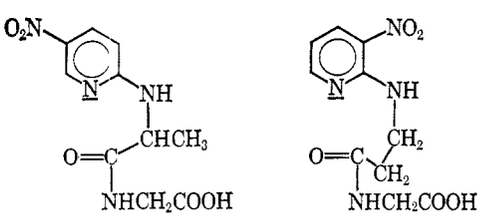
The acidity function H_0[1-3] has found wide application in the determination of the basicity of very weak bases and in the study of mechanism of certain acid-catalyzed reactions;[4] the H_0 function represents the ability of a solvent to donate a proton to a neutral base and in dilute solutions becomes identical with pH. Previous work from this laboratory[5] has shown that the acid hydrolysis of amide bond of 3,5-dinitro-2-pyridylalanylglycine (I) is an intramolecular-catalyzed process with participation of the protonated pyridyl group ($pK_a = -3.2$); the observed variations in the rate are proportional to the H_0 values with a slope of 1. Furthermore the striking fact that emerged from this earlier study, was that the results indicated a zero-order water dependency; this was demonstrated by determining the w value[6] for the hydrolysis of protonated I.

In view of these findings it is of considerable interest, principally for the possible applications in problems of selective hydrolysis of peptide linkages, to determine whether the catalytic properties of the pyridyl group are enhanced when the hydrolyses of 3-nitro-2-pyridylalanylglycine (II) and 5-nitro-2-pyridylalanylglycine (III) are considered. It can be assumed as a first approximation that the pK_a values of the heterocyclic nitrogen vary with the nature and number of nuclear substituents; in other words we make the assumption that the cyclic nitrogen atom in II and III is sufficiently basic to form appreciable quantities of kinetically important protonated species in dilute, aqueous acid solutions.



I

II



III

IV

On the other hand if the acid hydrolysis involves a cyclic intermediate through intramolecular nucleophilic displacement, the rates at which these reactions take place can, of course, depend markedly on the size of the ring that is formed; we have therefore examined the influence of the increasing length of the lateral chain performing kinetic measurements on 3-nitro-2-pyridyl-β-alanylglycine (IV).

The present communication records kinetic studies which lead to a quantitative comparison of these effects in terms of the velocity coefficients and Arrhenius parameters.

Experimental Section

Materials.—*dl*-Alanine and *dl*-alanylglycine are Fluka chromatographically pure products; they were tested for purity by chromatography on paper in at least two different solvent systems. 2-Chloro-3-nitropyridine, mp 102°, and 2-chloro-5-nitropyridine, mp 108°, were prepared by the action of $POCl_3$ in the presence of dimethylformamide[7] on the relative hydroxy compounds, and crystallized from methanol. However, fluoronitropyridines are preferable to chloro derivatives because they react at speeds conveniently more rapid for preparative purposes; for this reason starting from the chloro derivatives mentioned above, we have synthetized the relative fluoro compounds.[8]

Carbobenzoxy-β-alanylglycine Ethyl Ester.—The synthesis was performed according to Sheehan and Hess[9] by the following procedure. To a solution of 13.9 g of glycine ethyl ester hydrochloride (0.1 mole) and 14.4 ml of triethylamine (0.1 mole) in 150 ml of chloroform, 22.3 g of carbobenzoxy-β-alanine (0.1 mole) in 120 ml of chloroform and 30 ml of dimethylformamide, and 20.5 g of dicyclohexylcarbodiimide (0.1 mole) were added at 0°. The solution was then stirred overnight at room temperature. Dicyclohexylurea was removed by filtration and the filtrate was washed with 0.5 N hydrochloric acid, water, 5% sodium bicarbonate solution, and water, and finally dried over sodium sulfate. The solvent was evaporated *in vacuo;* crystallization from ethyl acetate–ether yielded 22.0 g (71%), mp 101°.[10]

β-Alanylglycine.—A solution of 8.1 g of carbobenzoxy-β-alanylglycine ethyl ester (0.026 mole) in 30 ml of acetone and 30 ml of 1 N sodium hydroxide was permitted to stand at room temperature for 30 min and then acidified with 5 N hydrochloric acid. After evaporation *in vacuo* of the acetone and crystallization from ethyl acetate–petroleum ether (bp 30–50°) carbobenzoxy-

(1) L. A. Flexser, L. P. Hammett, and A. Dingwall, *J. Am. Chem. Soc.*, **57**, 2103 (1935).
(2) H. Lemaire and H. J. Lucas, *ibid.*, **73**, 5198 (1951).
(3) C. D. Johnson, *et al.*, *Tetrahedron*, **21**, 1055 (1965).
(4) F. A. Long and M. A. Paul, *Chem. Rev.*, **57**, 935 (1957).
(5) A. Signor and E. Bordignon, *J. Org. Chem.*, **30**, 3447 (1965).
(6) J. F. Bunnett, *J. Am. Chem. Soc.*, **83**, 4956, 4973 (1961).
(7) A. Signor, L. Biondi, E. Scoffone, and S. Bezzi, *Gazz. Chim. Ital.*, **93**, 65 (1963).
(8) G. C. Finger and L. D. Starr, *J. Am. Chem. Soc.*, **81**, 2674 (1959).
(9) J. C. Sheehan and G. P. Hess, *ibid.*, **77**, 1067 (1955).
(10) N. T. Hanson and E. L. Smith, *J. Biol. Chem.*, **175**, 833 (1948).

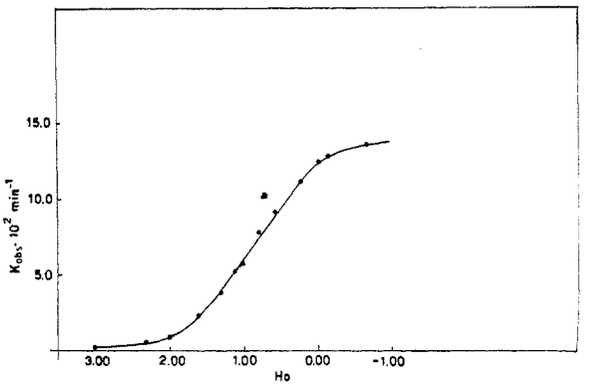


Figure 1.—The H_0 dependence of the hydrolysis of 3-NO_2-2-pyridylalanylglycine at 25°. Ionic strength maintained at 2.00 M for all reactions, through addition of NaCl.

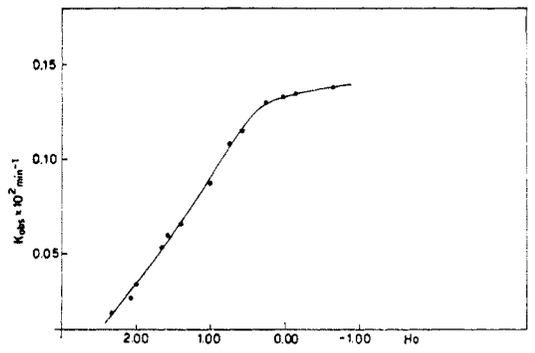


Figure 2.—The H_0 dependence of the hydrolysis of 5-NO_2-2-pyridylalanylglycine at 25°. Ionic strength maintained at 2.00 M for all reactions, through addition of NaCl.

β-alanylglycine had mp 148° (yield 90%).[10] Carbobenzoxy-β-alanylglycine (3.24 g) in methanol (100 ml) with a few drops of acetic acid added, rapidly adsorbed hydrogen in the presence of palladized charcoal. The free dipeptide was crystallized from water–acetone as monohydrate, mp 219°, yield 83%. *Anal.* Calcd for $C_5H_{10}N_2O_3 \cdot H_2O$: C, 36.50; H, 7.40; N, 17.08. Found: C, 36.54; H, 7.62; N, 17.20.

Nitro-2-pyridyl Derivatives.—The samples of 3- and 5-nitro-2-pyridyl derivatives used in this investigation were prepared in the following manner. A solution of 0.01 mole of amino acid or dipeptide and 2.0 g of sodium bicarbonate in 30 ml of water was mixed with a solution of 2.84 g of 2-fluoro-3 or -5-nitropyridine in 50 ml of ethanol and stirred vigorously for 2 hr at 40°. Ethanol and excess of 2-fluoronitropyridine were removed by repeated extractions with equal volumes of ether, on acidification to pH 2–3 with dilute hydrochloric acid, the compounds precipitate as yellow crystals.

3-Nitro-2-pyridyl-*dl*-alanine had mp 146–147°, yield 92%. *Anal.* Calcd for $C_8H_9N_3O_4$: C, 45.50; H, 4.26; N, 19.91. Found: C, 45.36; H, 4.40; N, 19.50.

3-Nitro-2-pyridyl-*dl*-alanylglycine had mp 185°, yield 93%. *Anal.* Calcd for $C_{10}H_{12}N_4O_5$: C, 44.77; H, 4.48; N, 20.89. Found: C, 44.52; H, 4.53; N, 20.64.

3-Nitro-2-pyridyl-β-alanylglycine had mp 159°, yield 95%. *Anal.* Calcd for $C_{10}H_{12}N_4O_5$: C, 44.77; H, 4.48; N, 20.89. Found: C, 44.64; H, 4.40; N, 20.55.

5-Nitro-2-pyridyl-*dl*-alanine had mp 170–171°, yield 89%. *Anal.* Calcd for $C_8H_9N_3O_4$: C, 45.50; H, 4.26; N, 19.91. Found: C, 45.22; H, 4.33; N, 19.62.

5-Nitro-2-pyridyl-*dl*-alanylglycine had mp 144–145°, yield 88%. *Anal.* Calcd for $C_{10}H_{12}N_4O_5$: C, 44.77; H, 4.48; N, 20.89. Found: C, 44.05; H, 4.67; N, 20.48.

Kinetic Procedure.—The kinetics of hydrolysis of II, III, and IV were determined by following the appearance of glycine by the method of Spackman, Moore, and Stein.[11] The mechanical procedures employed in performing the rate determinations are described in an earlier paper.[5]

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

pK_a Determination.—The ionization constants of II and III were determined by the spectrophotometric method described in a previous paper,[5] hydrochloric acid being used instead of sulfuric acid. All spectral measurements were made with a Model DU Beckman spectrophotometer at 25° and at constant ionic strength; the appropriate solvent was used as a blank for each determination. Acids of known concentration were used to make up to the mark, and the H_0 of the subsequent solutions were obtained by interpolation from the figures of Paul and Long.[12] The H_0 data are reported without correction for the neutral salt added; it is well known,[13] however, that the neutral salt effect on H_0 at the lower HCl concentrations is at least impressively linear with respect to the salt concentration. Furthermore at relatively low salt concentration, the corrections are small.

Results

The hydrolyses of II and III in acid solution are accurately first order over at least 90% of the course of reactions; the accuracy of the first-order constants obtained is ±5%. The reactions have been studied at temperatures of 25, 35, 45, and 60 ± 0.1° for solutions of amides in water, maintaining constant ionic strength by addition of suitable amounts of sodium chloride; we have observed that the rate constants are markedly affected by ionic strength. The stoichiometry of these reactions demands that for each mole of nitro-2-pyridylalanylglycine cleaved, 1 mole of nitro-2-pyridylalanine and 1 mole of glycine are formed. In all cases the substrate concentration was in the range of 0.001–0.005 mole/l. Table I gives a summary of the experimental data for the reactions at 25°.

TABLE I

RATE CONSTANTS FOR THE ACID HYDROLYSIS OF II AND III IN WATER AT 25°[a]

HCl concn, M	H_0[b]	$k_{obsd} 10^2$, min^{-1}
3-Nitro-2-pyridylalanylglycine		
0.001	+3.00	0.16
0.005	+2.30	0.50
0.010	+2.00	0.76
0.024	+1.61	2.35
0.047	+1.31	3.70
0.073	+1.12	5.10
0.095	+1.00	5.60
0.142	+0.80	7.80
0.245	+0.56	9.10
0.492	+0.21	11.10
0.740	−0.03	12.50
0.937	−0.16	12.80
1.980	−0.68	13.50
5-Nitro-2-pyridylalanylglycine		
0.005	+2.30	0.019
0.009	+2.04	0.026
0.010	+2.00	0.032
0.022	+1.65	0.054
0.040	+1.39	0.066
0.100	+0.98	0.087
0.158	+0.76	0.108
0.257	+0.54	0.115
0.474	+0.23	0.130
0.675	+0.02	0.133
0.910	−0.15	0.135
1.880	−0.64	0.138

[a] NaCl was added to make ionic strength 2.00 M. [b] The data of H_0 are derived from Paul and Long.[12]

(12) M. A. Paul and F. A. Long, *Chem. Rev.*, **57**, 1 (1957).
(13) M. A. Paul, *J. Am. Chem. Soc.*, **76**, 3236 (1954).

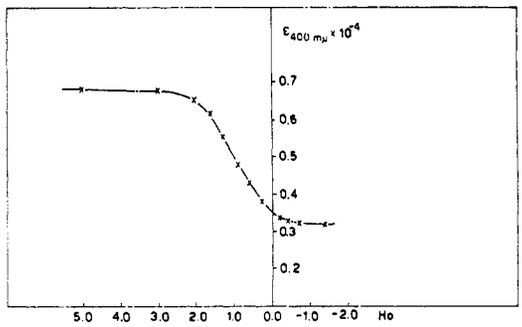


Figure 3.—3-NO_2-2-Pyridylalanylglycine; ϵ at 400 mμ against H_0. Points × are experimental results; line calculated from $pK_a = H_0 + 1.014 \times \log C_{BH^+}/C_B$.

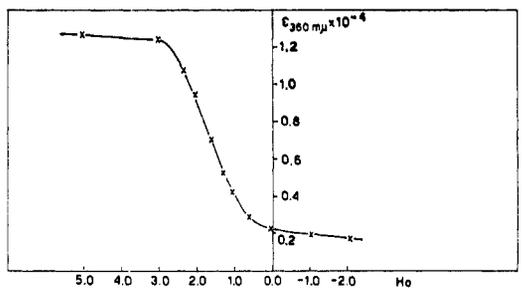


Figure 4.—5-NO_2-2-Pyridylalanylglycine; ϵ at 360 mμ against H_0. Points × are experimental results; line calculated from $pK_a = H_0 + 1.050 \times \log C_{BH^+}/C_H$.

The point of greatest interest with these hydrolyses is the correlation of the rate of the acid reactions with H_0; this is illustrated by Figures 1 and 2 which give a plot of rate constants for the two substrates against H_0. To evaluate the possible connection between acidity of the acids and their respective rates of hydrolysis and in order to establish if II and III are Hammett bases we have investigated in detail the protonation behavior of such compounds; this study required knowledge of the relative concentrations of free base and conjugate acid present at known H_0. We have operated at wavelengths where there is a large change from cation to neutral molecule; graphs of extinction coefficients against H_0 showed the usual sigmoid-type curves (Figures 3 and 4).

The values of n and pK_a in eq 1 were obtained by

$$H_0 = pK_a - n \log C_{BH^+}/C_B \quad (1)$$

plotting $\log C_{BH^+}/C_B$ against H_0. The former quantity was obtained from

$$C_{BH^+}/C_B = \frac{\epsilon_B - \epsilon}{\epsilon - \epsilon_{BH^+}}$$

where ϵ, ϵ_B, and ϵ_{BH^+} have their usual significance. We found that $n = 1.01$ and $pK_a = +0.99$ for II and $n = 1.05$ and $pK_a = +1.65$ for III.

The effect of temperature on the hydrolyses of II and III as measured by the rate of appearance of glycine is given in Table II. An Arrhenius plot was constructed from the data of Table II to determine the activation energies. Figure 5 shows that the activation energies are reasonably constant over the temperature range studied. The apparent activation energy for the hydrolysis of II is +18.07 kcal/mole; the entropy of activation, calculated from the Eyring equation, is −10.95 eu. The values for III are +20.27 kcal/mole and −12.32 eu, respectively.

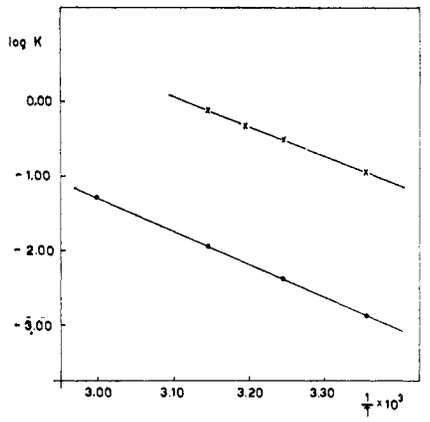


Figure 5.—Log k against $1/T$ plotted between 25 and 60° for 3-NO_2-2-pyridylalanylglycine (×), E = +18.07 kcal/mole and log A = 10.51; for 5-NO_2-2-pyridylalanylglycine (●), E = +20.27 kcal/mole and log A = 10.21.

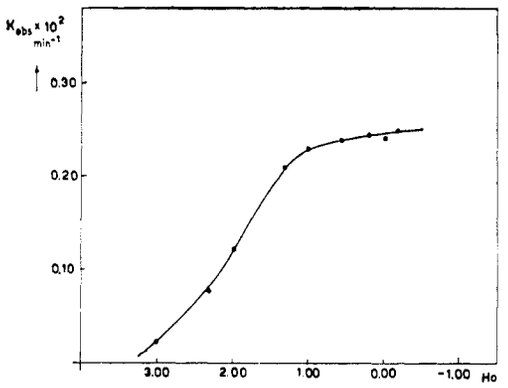


Figure 6.—The H_0 dependence of the hydrolysis of 3-NO_2-2-pyridyl-β-alanylglycine at 60°. Ionic strength maintained at 2.00 M for all reactions, through addition of NaCl.

TABLE II

TEMPERATURE EFFECT ON THE ACID-CATALYZED HYDROLYSES OF 3-NITRO-2-PYRIDYLALANYLGLYCINE (II) AND 5-NITRO-2-PYRIDYLALANYLGLYCINE (III)[a]

	$k_{obsd}10^3$, min^{-1}	
Temp, °C	II	III
25	11.10	0.13
35	29.00	0.43
40	48.40	...
45	73.50	1.09
60	...	4.95

[a] In all experiments the hydrochloric acid concentration was 0.472 M; ionic strength constant 2.00 M was provided by NaCl.

These results suggest that in favorable systems pyridine catalysis of the hydrolysis of carboxylic acid derivatives can occur. It was possible to demonstrate this catalytic effect also for the acid hydrolysis of IV in which there is elongation of the side chain; in Figure 6 the variation of the observed rate constants of hydrolysis as a function of H_0 can be seen with good evidence. There is however a marked decrease in the rate constants in comparison with the parent compound II; the experimental data obtained at 60° are recorded in Table III.

Discussion

The shapes of the H_0-dependence curve for II, III, and IV are in excellent agreement with the participation of only the protonated pyridyl group; the varia-

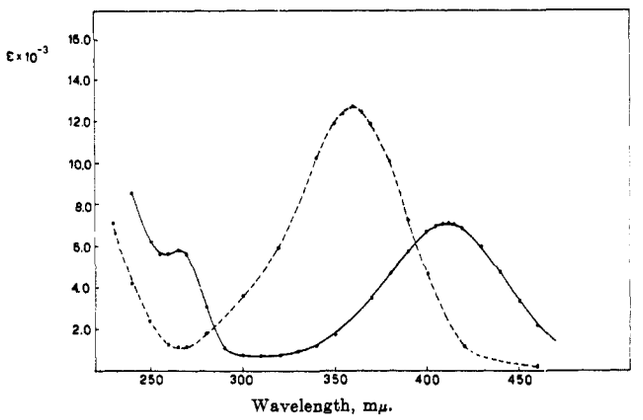


Figure 7.—Light absorption curves for 3-NO_2-2-pyridylalanylglycine (solid line) and 5-NO_2-2-pyridylalanylglycine (broken line) in 1% $NaHCO_3$.

TABLE III

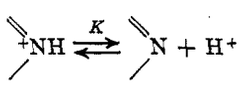
RATE CONSTANTS FOR THE ACID HYDROLYSIS OF IV IN WATER AT 60°[a]

HCl concn, M	$k_{obsd}10^2$, min^{-1}
0.001	0.0220
0.005	0.0778
0.010	0.1220
0.050	0.2080
0.100	0.2300
0.250	0.2380
0.500	0.2440
0.751	0.2400
1.010	0.2530

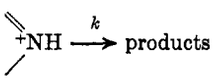
[a] NaCl was added to make ionic strength 2.00 M.

tion of the rate constant from $H_0 = -1.0$ to $+3.0$ indicates kinetic dependence on the conjugate acids of the nitropyridyl derivatives and independence of the external hydrogen ion concentration. This H_0 dependence is characterized by constancy of the rate constants from $H_0 = -1.0$ to 0.0 and a decrease in the rate constants from $H_0 = 0.0$ to $+3.0$; therefore we assume that the H_0 profiles between -1 and $+3$ for these reactions follow the ionization curves for the basic heterocyclic nitrogen group, the rates being proportional to the concentration of the ionized form.

With reference to this hypothesis if two assumptions are made, to wit



and



it can be shown that

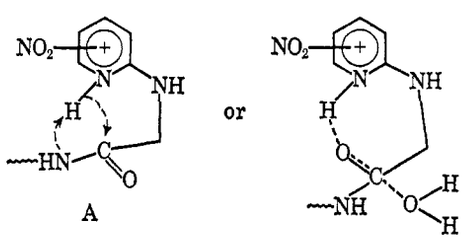
$$1/k_{obsd} = 1/k + K/k \times 1/h_0 \qquad (2)$$

where K is the equilibrium constant, k is the specific rate constant, and h_0 the antilogarithm of H_0 (which in dilute acid solution is identical with a_{H^+}). According to eq 2 the kinetic dependence on protonated pyridine species is quantitatively confirmed by the linearity of a plot of $1/k_{obsd}$ *vs.* $1/h_0$; the values of pK_a and k derived from such a plot (least squares) are $+0.89$ and 0.133 min^{-1} for II and $+1.56$ and 0.00122 min^{-1} for III, respectively. As can be seen the apparent equilibrium constants K computed indirectly from kinetic data have values close to those determined by spectrophotometric method. A satisfactory linear plot of $1/k_{obsd}$ against $1/h_0$ was also obtained for IV; from intercept and slope we have calculated the values of $k = 0.00246$ min^{-1} and $pK_a = +0.91$. This latter result for the equilibrium constant is in excellent agreement with that obtained for the acid hydrolysis of II.

That the protonated pyridyl group assists in amide hydrolysis finds a parallel in the catalysis of amide hydrolysis by participation of the undissociated carboxyl group[14-16] and of the protonated imidazolyl group.[17]

Furthermore this result is in accord with the previous demonstration of the catalytic role of pyridine in the hydrolysis of 3,5-dinitro-2-pyridylalanylglycine (I) in strongly acid aqueous solutions; the resistance of the amide bond in I to acid hydrolysis is appreciably greater than that of II and III, as expected from the electron-withdrawing properties of two nitro groups which markedly decrease the basicity of the cyclic nitrogen atom.

The assistance of the pyridyl group in the acid hydrolysis of II and III can be rationalized by postulating either a four-center electrophilic–nucleophilic mechanism or an intramolecular solvation of the transition state for water attack by the pyridinium ion; these two possibilities cannot be differentiated kinetically (eq 3).



(3)

Both mechanisms involve a preliminary acid–base equilibrium; in the concerted electrophilic–nucleophilic mechanism this is followed by a rate-determining unimolecular rearrangement to give an acylpyridinium ion. The water molecule then adds in a follow reaction. The other mechanism differs in that the water molecule is involved in the rate-determining step.

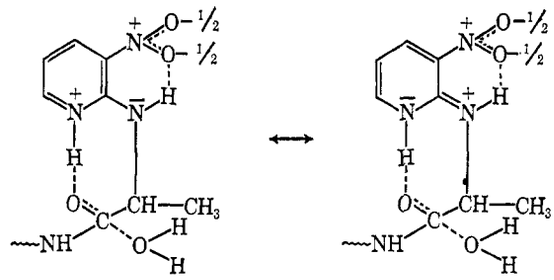
We feel quite confident that B is the transition state for the hydrolysis as will be apparent later on the basis of steric arguments.

The explanation of the large difference in the reaction rates between 3- and 5-nitro-2-pyridylalanylglycine (differing by a factor of *ca.* 100) is by no means simple. From Table II it can be seen that there is a remarkable decrease in the activation energy while no appreciable difference exists between the activation entropies. A satisfactory explanation can be achieved if appropriate spatial orientation of substrate bond to catalytic site is taken in account. As will appear later, there is no doubt that in 3-nitro-2-pyridylalanylglycine there is a considerable steric inhibition of the mesomerism; this steric strain is revealed by the lowered basic strength

(14) M. L. Bender, Y. Chow, and F. Chloupek, *J. Am. Chem. Soc.*, **80**, 5380 (1958).
(15) M. L. Bender, F. Chloupek, and M. C. Neveu, *ibid.*, **80**, 5384 (1958).
(16) S. J. Leach and H. Lindley, *Trans. Faraday Soc.*, **49**, 921 (1953).
(17) T. C. Bruice and J. M. Sturtevant, *J. Am. Chem. Soc.*, **81**, 2860 (1959).

as compared with 5-nitro-2-pyridylalanylglycine. However, a clearer picture of the effect of steric hindrance is presented in Figure 7 in which are reported the ultraviolet spectra of II and III in 1% $NaHCO_3$; the absorption of *ortho* compound II (ϵ_{max} 7100) is less intense than that of the *para* isomer III (ϵ_{max} 12,700). It is well known that the most general consequence of nonplanarity on electronic spectra is a decrease in the absorption intensity of the so-called *K* bands, *i.e.*, absorption bands which owe their existence to the presence of fully extend conjugation. This decrease in absorption intensity may be accompanied, by a shift of the absorption maximum (λ_{max}) either to shorter or to longer wavelengths; in particular the changes in λ_{max} to longer wavelengths, as in the present case, may be expected for molecules in which excitation is attended by a decrease of double-bond character. Studies with *ortho*-substituted anilines and dimethylanilines have shown similar effects;[18,19] the absorption intensity for the 400-mμ band of *o*-nitroaniline is much less intense than for the *para* derivative and there is also some change in absorption maximum.

Therefore it is reasonable to assume that in the ground state there is no possibility of hydrogen bonding in a planar structure for II; on the other hand, geometrical considerations make it probable that in the transition state the *o*-nitro group and the secondary amino nitrogen are coplanar with the pyridine ring. We propose, as a tentative hypothesis, that in this situation hydrogen bond occurs with a contribution to the hybrid from the following canonical structures (eq 4). The transition state is thereby stabilized and the energy barrier is lowered. The energy of formation of the N—H···O bond is ≃2000 cal,[20] so that this



(4)

factor alone could entirely account for the differences observed in Arrhenius parameters. In the case of the acid hydrolysis of III, it is improbable that steric hindrance is significant and furthermore there is no possibility of intramolecular hydrogen bond; this suggests very strongly that the *o*-nitro group is largely responsible for the steric compression with the two other substrates (II and IV) and for the great enhancement of the rate constants for II. The remarkable stability of IV toward hydrolysis is noteworthy; we would like to suggest that the stability of this compound to acid-catalyzed hydrolysis is probably due to a lack of steric requirements for the formation of the cyclic transition state.

The foregoing results strongly support the view that the neighboring heterocyclic nitrogen plays an important role in the cleavage of peptide bonds under hydrolytic acid conditions.

Registry No.—β-Alanylglycine, 2672-88-0; 3-nitro-2-pyridyl-*dl*-alanylglycine, 7594-53-8; II, 7594-54-9; IV, 7594-55-0; 5-nitro-2-pyridyl-β-alanylglycine, 7594-56-1; III, 7594-57-2.

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(18) G. Glotz, *Bull. Soc. Chim. France*, **1**, 1148 (1934).
(19) W. R. Remington, *J. Am. Chem. Soc.*, **67**, 1838 (1945).
(20) M. Davies, *Ann. Rept. Progr. Chem.*, **43**, 5 (1946).

Synthesis of Pyridazine Derivatives. XIV. Polyaza Heterocycles Derived from Pyrido[2,3-*d*]pyridazine[1]

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The synthesis of polyaza heterocycles starting from pyrido[2,3-*d*]pyridazines is described. Treatment of I or VIII (R = H) with bromoacetaldehyde, bromoacetone, or phenacyl bromide afforded the isomeric imidazo[1,2-*b*]pyridopyridazines (II and IX, R = H, CH_3, C_6H_5). Similarly, the corresponding hydrazino-substituted products (I or VIII, R = NH_2) were employed for the preparation of the isomeric fused tetrazolo- or triazoloaza heterocycles (VI and VII and XIII and XIV). Hydrazinolysis of II (R = H) or IX gave the isomeric hydrazino-substituted compounds III (R_1 = R_2 = H) and X (R_1 = R_2 = H) from which in two different ways the isomeric tetracyclic imidazo[1,2-*b*]pyrido-*s*-triazolo[3,4-*f*]pyridazines (IV and XI) or their derivatives (V and XII) were obtained.

Continuing our investigations on condensed nitrogen-containing heterocyclic systems,[2-4] it was of interest to prepare some polyaza heterocycles with bridgehead nitrogen or nitrogens as the first step toward further investigations of their reactivity. In the present series, properly substituted pyrido[2,3-*d*]pyridazines were employed as starting materials and through a combination of this ring system with imidazole, triazole, or tetrazole rings, there were obtained several so far unknown parent isomeric polyaza heterocycles.

The chemistry of pyrido[2,3-*d*]pyridazines was developed only recently[5-8] and lately also both isomeric

(1) Paper L on Heterocycles.
(2) A. Pollak and M. Tišler, *Tetrahedron*, **22**, 2073 (1966).
(3) B. Stanovnik and M. Tišler, *Tetrahedron Letters*, 2403 (1966).
(4) B. Stanovnik and M. Tišler, *Tetrahedron*, **23**, 387 (1967).
(5) Y. Nitta, I. Matsuura, and F. Yoneda, *Chem. Pharm. Bull.* (Tokyo), **13**, 586 (1965).
(6) W. L. F. Armarego, *J. Chem. Soc.*, 6073 (1963).
(7) F. Bottari and S. Carboni, *Gazz. Chim. Ital.*, **86**, 990 (1956).
(8) Japanese Patent 22,266 (1965); *Chem. Abstr.*, **64**, 3567 (1966).